From genes to communities: Assessing plant diversity and connectivity in kettle holes as metaecosystems in agricultural landscapes

Sissi Donna Lozada Gobilard

Univ.-Diss.
zur Erlangung des akademischen Grades
"doctor rerum naturalium"
(Dr. rer. nat.)
in der Wissenschaftsdisziplin "Botanik"

eingereicht als publikationsbasierte Dissertation an der
Mathematisch-Naturwissenschaftlichen Fakultät
Institut für Biochemie und Biologie
der Universität Potsdam

Ort und Tag der Disputation: Potsdam, 16. Oktober 2019
Hauptbetreuer/in: Prof. Dr. Jasmin Joshi
weitere Gutachter: Prof. Dr. Catrin Westphal, Prof. Dr. Ulrike Herzschuh

"An understanding of the natural world and what's in it is a source of not only a great curiosity but great fulfillment. "

Sir David Attenborough

Abstract

Species assembly from a regional pool into local metacommunities and how they colonize and coexist over time and space is essential to understand how communities response to their environment including abiotic and biotic factors. In highly disturbed landscapes, connectivity of isolated habitat patches is essential to maintain biodiversity and the entire ecosystem functioning. In northeast Germany, a high density of the small water bodies called kettle holes, are good systems to study metacommunities due to their condition as "aquatic islands" suitable for hygrophilous species that are surrounded by in unsuitable matrix of crop fields. The main objective of this thesis was to infer the main ecological processes shaping plant communities and their response to the environment, from biodiversity patterns and key life-history traits involved in connectivity using ecological and genetic approaches; and to provide first insights of the role of kettle holes harboring wild-bee species as important mobile linkers connecting plant communities in this insular system.

At a community level, I compared plant diversity patterns and trait composition in ephemeral vs. permanent kettle holes). My results showed that types of kettle holes act as environmental filers shaping plant diversity, community-composition and trait-distribution, suggesting species sorting and niche processes in both types of kettle holes. At a population level, I further analyzed the role of dispersal and reproductive strategies of four selected species occurring in permanent kettle holes. Using microsatellites, I found that breeding system (degree of clonality), is the main factor shaping genetic diversity and genetic divergence. Although, higher gene flow and lower genetic differentiation among populations in wind vs. insect pollinated species was also found, suggesting that dispersal mechanisms played a role related to gene flow and connectivity. For most flowering plants, pollinators play an important role connecting communities. Therefore, as a first insight of the potential mobile linkers of these plant communities, I investigated the diversity wild-bees occurring in these kettle holes. My main results showed that local habitat quality (flower resources) had a positive effect on bee diversity, while habitat heterogeneity (number of natural landscape elements surrounding kettle holes 100–300m), was negatively correlated.

This thesis covers from genetic flow at individual and population level to plant community assembly. My results showed how patterns of biodiversity, dispersal and reproduction strategies in plant population and communities can be used to infer ecological processes. In addition, I showed the importance of life-history traits and the relationship between species and their abiotic and biotic interactions. Furthermore, I included a different level of mobile linkers (pollinators) for a better understanding of another level of the system. This integration is essential to understand how communities respond to their surrounding environment and how disturbances such as agriculture, land-use and climate change might affect them. I highlight the need to integrate many scientific areas covering from genes to ecosystems at different spatiotemporal scales for a better understanding, management and conservation of our ecosystems.

Zusammenfassung

Die Zusammenstellung regionaler Artgemeinschaften in eine lokale Metagemeinschaft ist essentiell für das Verständnis artspezifischer Reaktionen auf ihre biotische und abiotische Umwelt als auch, wie sie diese in zeitlichem und räumichem Umfang besiedeln und koexistieren. In fragmentierten Landschaften ist die Verknüpfung isolierter Habitate (Konnektivität) nötig, um die Biodiversität und Funktionalität von Ökosystemen aufrecht zu erhalten. Der Nordosten Deutschlands ist durch eine hohe Dichte von Kleinstgewässern, die solch isolierte Habitate darstellen, charakterisiert. In einer Matrix aus Agrarfeldern dienen diese sogenannten Sölle aquatischen Arten als „Habitatsinsel". Aufgrund dieser Landschaftsstruktur stellen sie ein geeignetes Untersuchungsgebiet für Metagemeinschaften dar. Das Ziel diser Arbeit ist es ökologische Prozesse zu untersuchen, die zur Vegetationszusammensetzung und deren Reaktion auf sich ändernde Umweltbedingungen führen. Mittels ökologscher und genetischer Methoden wird dies auf der Grundlage von Biodiversitätsmustern und Lebenszyklusmerkmalen untersucht, die in die Konnektivität involviert sind. Auf Pflanzengemeinschaftsebene wurden Diversitätsmuster und Merkmalszusammensetzungen in ephemeren und permanenten Söllen verglichen. Die Ergebnisse zeigen, dass die unterschiedlichen Typen von Söllen als Umweltfilter agieren, die die pflanzliche Artenvielfalt, Gemeinschaftszusammensetzung und Merkmalsverteilung beeinflussen. Dies führt zu der Schlussfolgerung, dass „Species-sorting" und Prozesse der Nichenbildung in beiden Typen von Söllen vorkommen. Auf Populationsebene wird der Ausbreitungsmeachnismus sowie die Reproduktionsstrategie vier verschiedener Pflanzenarten untersucht. Durch Mikrosatellitenanalysen wird gezeigt, dass der Grad der Klonalität den größten Einfluss auf die genetischen Diversität und den Genfluss hat. Zusätzlich weisen molekulare Analysen auf ein geringes Maß an genetischen Unterschieden zwischen Populationen windbestäubter Arten im Vergleich zu insektenbestäubter Arten hin. Dies bedeutet, dass der Ausbreitungsmechanismus einer Art einen grundlegenden Einfluss auf den Genfluss und die Konnektivität von Populationen hat. Für viele blühende Pflanzen, spielen Bestäuber, wie Wildbienen, eine wesentliche Rolle bei der Vernetzung isolierter Habitate. Um das Potential dieser mobilen Linker zu untersuchen, wird die Wildbienendiversität verschiedener Sölle analysiert. Dadurch konnte gezeigt werden, dass die lokale Habitatsqualität (Blütenressourcen) einen positiven Effekt auf die Artenvielfalt hat, während die Habitatsheterogenität (Anzahl von natürlichen Landschaftselementen in unmittelbarer Nähe) eine negative Korrelation aufweist. Diese Ergebnisse unterstreichen die Bedeutung von Wildbienenpopulationen als mobile Linker zwischen isolierten Habitaten. Die Ergebnisse dieser Arbeit zeigen, wie durch Biodiversitätsmuster, Verbreitungs- und Reproduktionsstrategien pflanzlicher Gemeinschaften auf ökologische Prozesse rückgeschlossen werden kann. Des Weiteren ist die Wichtigkeit der Lebenszyklusmerkmale zwischen Arten und deren Umweltinteraktionen verdeutlicht. Die Berücksichtigung mobiler Linker (Bestäuber) ermöglicht eine zusätzliche Betrachtungsebene. Durch diese Arbeit wird die Notwendigkeit hervorgehoben, verschiedene wissenschaftliche Bereiche, wie Genetik und Ökologie, zu vereinen, um ein allumfassendes Verständnis unserer Ökosysteme zu erlangen und somit zu ihrem Schutz beizutragen.

Resumen

El ensamblaje de metacomunidades a partir de un grupo regional de especies es esencial para entender cómo las especies responden a su medio ambiente (abiótico y biótico) y cómo colonizan y coexisten en el tiempo y el espacio. En paisajes altamente fragmentados, la conectividad entre parches de hábitat aislados es necesaria para mantener la biodiversidad y el funcionamiento de ecosistemas. En el noreste de Alemania, una alta densidad de pequeños cuerpos de agua llamados *kettle holes*, son sistemas ideales para estudiar metacomunidades debido a su condición de islas acuáticas rodeadas por una matriz inadecuada de campos de cultivo, adecuadas para hospedar especies higrófilas. En este sentido, el objetivo principal de esta tesis fue inferir los principales procesos ecológicos que conforman las comunidades locales de plantas y su respuesta al medio ambiente, a partir de patrones de biodiversidad, relacionando rasgos funcionales clave y conectividad a diferentes niveles con enfoques ecológicos y genéticos. A nivel de comunidades de plantas, se comparó los patrones de diversidad y composición de rasgos funcionales en dos tipos de *kettle holes*: efímeros o temporales vs. permanentes. Mis resultados sugieren que el tipo de *kettle holes* actúa como filtros ambientales estructurando la diversidad de plantas, la composición de la comunidad y la distribución de rasgos funcionales. Estos resultados podrían ser una indicación de procesos de clasificación de especies (“*Species Sorting*”) y de diferenciación de nicho en ambos tipos de pozos. A nivel poblacional, analicé el rol de la dispersión y las estrategias reproductivas de cuatro especies seleccionadas distribuidas en estos ecosistemas acuáticos de tipo permanente. Usando microsatélites, descubrí que el grado de clonalidad es el factor principal que determina la diversidad genética y el flujo de genes en dichas especies. Sin embargo, también se encontró un mayor flujo de genes y una menor diferenciación genética entre las poblaciones en las especies polinizadas por el viento frente a los insectos, lo que sugiere que los mecanismos de dispersión podrían tener un papel menor relacionado con el flujo de genes y la conectividad. Para la mayoría de las plantas con flores, los polinizadores desempeñan un papel importante en la conexión de las comunidades transportando genes y coadyuvando a la reproducción. Por lo tanto, como una primera idea de los posibles “*mobile linkers*” de estas comunidades de plantas, también investigué la diversidad de abejas silvestres en estos pozos de agua de tipo permanente. Mis principales resultados mostraron que la calidad del hábitat (recursos florales) tuvo un efecto positivo en la diversidad de abejas, mientras que la heterogeneidad del hábitat (número de elementos del paisaje natural que rodea los *kettle holes*) tuvo un efecto negativo. En general, los resultados mostraron cómo los patrones de biodiversidad, dispersión y estrategias de reproducción en poblaciones y comunidades de plantas pueden usarse para inferir procesos ecológicos. También mostré la importancia de los rasgos funcionales respecto a las interacciones abióticas y bióticas. Además, incluí un nivel diferente de “*mobile linkers*” (polinizadores) para una mejor comprensión del sistema. La integración de múltiples niveles es esencial para comprender cómo las comunidades responden a su entorno y a las perturbaciones como la agricultura, el uso de la tierra y el cambio climático. Resalto la necesidad de integrar distintas áreas científicas que abarquen desde genes hasta ecosistemas a diferentes escalas espaciotemporales para un mejor manejo y conservación de nuestros ecosistemas.

Contents

CHAPTER 1

General introduction

1.1 Community assembly

For many years, ecologists have been interested in studying communities. Community ecology focuses on understanding how species assemble from a regional pool into local communities, sorted by different environmental (abiotic and biotic) filters and how they successfully colonize and coexist over time and space (Weiher and Keddy 1995; HilleRisLambers *et al.* 2012; Mittelbach and Schemske 2015; Zobel 2016). Many abiotic and biotic factors can influence assembly of local communities at different spatial-temporal scales. For example, relative fitness, demographic drift, dispersal and niche differences between species and their environment (HilleRisLambers *et al.* 2012); as well as their phylogenetic relationships, geography of speciation and local adaptation (Emerson and Gillespie 2008; Mittelbach and Schemske 2015). Therefore, the combination of community ecology with other disciplines such as evolutionary biology and biogeography, is needed to fully understand community assembly (Götzenberger et al. 2012; Zobel 2016).

Human activities, such as agriculture or deforestation can cause habitat fragmentation impacting natural communities over short-time scales (Venter *et al.* 2016). Economic growth and constant demand for food causes conversion of landscapes into agricultural fields generating biodiversity loss and ecosystem degradation worldwide (Liu *et al.* 2018; Marques *et al.* 2019). Crop and livestock farming are the main activities causing habitat fragmentation and species loss (Maxwell *et al.* 2016). In Europe, more than 45% of the land is used for agricultural activities and 52% of the land surface in Germany is used for intensive agriculture, affecting the biodiversity, water quality and soil (Ramankutty *et al.* 2008; Gutzler *et al.* 2015).

1.1.1 Metacommunity theory and the four paradigms

Habitat fragmentation can cause reduction of local communities due to changes in the abiotic conditions and biotic interactions, resulting in isolated patches (Lienert 2004). These patches are more vulnerable to a decrease in population survival, individual fitness, genetic diversity and gene flow (Lienert 2004; Leimu *et al.* 2006). When isolated set of local communities are linked by dispersal of multiple interacting species, they are regarded as “metacommunities”, and are also influenced by local interactions and regional processes (Wilson 1992; Leibold *et al.* 2004; Logue *et al.* 2011).

TABLE 1.1: **Conceptual paradigms of metacommunity theory.** Four paradigms of metacommunities where the first three show differences in dispersal and heterogeneity of habitat patches; while neutral model highlight that community assembly depends entirely on demographic stochasticity without differences in fitness or niche. Concepts taken from Logue *et al.* (2011).

Paradigm	Habitat patches	Dispersal
Species Sorting (SS)	Heterogeneous	High to enable species to coexist by means of niche diversification and differences in resource exploitation.
Mass Effects (ME)	Heterogeneous	High to enable reproduction in a source habitat that allows for persistence within a sink habitat.
Patch dynamics (PD)	Homogeneous	Differ among species. A colonisation– competition trade-off occurs, with successful colonisers outcompete poor competitors.
Neutral model (NM)	Species do not differ in their fitness or niche (i.e., species composition within habitat patches is not driven by differences in competitiveness or mobility).	

In order to identify the most important ecological processes in metacommunities, Leibold *et al.* (2004) have proposed four paradigms based on species characteristics (mainly dispersal), and environmental conditions: Species Sorting (SS), Mass Effects (ME), Patch Dynamics (PD) and Neutral Model (NM) revised by Logue *et al.* (2011). In a Neutral Model (NM) processes are stochastic and species do not differ in fitness or niche. Patch Dynamics (PD) assumes homogeneous patches where species differ in their dispersion and colonization-competition trade-off. In Species Sorting (SS) and Mass Effects (ME), environmental heterogeneity plays a role in filtering species due to niche difference (in case of SS) or due to a source-sink mechanism when patches are interconnected (in case of ME) (Table 1.1).

However, these four metacommunity paradigms are not exclusive and do not represent the complete set of metacommunity dynamics (Leibold *et al.* 2004; Brown *et al.* 2017). Both, biotic and abiotic interaction can influence community assembly (Gross *et al.* 2013) and interactions between individuals within a community can be positive (e.g. mutualistic) or negative (e.g. competitive) (Brooker and Callaghan 1998). Additionally, specific details of abiotic features such as metacommunity sizes, degree of isolation or scale of heterogeneity are important variables to consider as well (Brown *et al.* 2017).

1.1.2 Size and proximity: two important variables in metacommunities

In general, species richness is positively related with the size of a suitable patch (e.g., area), but negatively related to isolation (e.g., distance to the closest habitat). On the one hand, the positive island species-area relationship can be generally explained by the framework of "environmental heterogeneity" where a wider range of habitats is suitable for more different plant species in larger habitats (Stein *et al.* 2014). On the other hand, the negative relationship between isolation and species richness is based on the higher probability of dispersal and colonization to a closer island (MacArthur and Wilson 1967). These two concepts are based on the the island biographic theory (MacArthur and Wilson 1967). This model assumes a binary system of islands (discrete isolated habitat patches) and inhospitable matrix where connectivity is determined by the size and proximity (MacArthur and Wilson 1967; McGarigal *et al.* 2005).

Island-like habitats can be regarded as "metaecosystems". The concept of metaecosystem was proposed by Loreau *et al.* (2003), was defined as a set of ecosystems connected by spatial flows of energy, materials and organisms across ecosystem boundaries. When organisms disperse connecting metacommunities, they modify habitats and consequently the functioning of the ecosystem due to flow of resources (Gounand *et al.* 2018). In this metaecosystem dynamic, different types of movements or processes (e.g. predation, biomass recycling, mating aggregations) are involved in the coupling of ecosystems (Gounand *et al.* 2018).

From an evolutionary perspective, after a long time, large "islands" should possess a higher probability of speciation via cladogenesis and in this case, isolation is expected to be less important for species richness in larger islands (Weigelt and Kreft 2013). Trophic, competitive, facilitative or mutualistic interactions, dispersal limitation, as well as evolutionary dynamics, can operate simultaneously shaping community assembly (e.g., Brooker and Callaghan 1998; Brooker *et al.* 2007; Spasojevic and Suding 2012; Schöb *et al.* 2014). Therefore, more recent models highlight the ecological and evolutionary feedbacks between local (meta)communities and regional species pool (Mittelbach and Schemske 2015).

1.1.3 Phylogenetic relationships and functional traits

Phylogenetic relationships have been used to infer the assembly of communities. Based on the idea that morphological and ecological similarity between species is related to phylogenetic relatedness, dispersed communities are the result of competition of related taxa; while clustered ones are the result of environmental filtering processes (Webb *et al.* 2002; Cahill *et al.* 2008). Therefore, this approach has been used to identify and separate biotic interactions from environmental filtering. However, there is not often a relationship between ecological and phylogenetic similarities and therefore phylogenetic relationships alone are not good indicators for community assembly mechanisms (Losos 2008; Cavender-Bares *et al.* 2009; Mayfield and Levine 2010; Gerhold *et al.* 2015; Cadotte *et al.* 2017).

Functional traits can also be used to infer the effect of biotic and abiotic processes on community assembly. Functional traits are all morphological characteristics related to a ecophysiology response of an organism to environmental factors (Ottaviani *et al.* 2016; Wittmann *et al.* 2016). For instance, specific leaf area (SLA), plant height or wood width are important traits to predict species distribution and communities' composition and the response to their environment (McGill *et al.* 2006). These functional traits could be interpreted as the result of biotic interactions (intraspecific competition) or environmental filtering similarly to the phylogenetic relationships (Kraft *et al.* 2007, 2008). Many studies have shown that combining phylogenetic relationships with life-history traits are useful to predict how species and communities will respond to their environment, for example to climate change (e.g., Willis *et al.* 2008; Comte *et al.* 2014).

1.1.4 Connectivity between metacommunities

Movement is very important not only to understand how metacommunities assemble but also the entire functioning of ecosystems. Unlike animals, plants are sessile, possessing a passive mode of dispersal and a strong spatial structure. However, processes such as colonization, re-colonization and extinction essential for metacommunity dynamics are very difficult to measure (Watkinson and Freckleton 2002; Husband and Barret 2009). To colonize new suitable patches, plants rely on passive dispersal of pollen and seeds mediated through abiotic (wind, water) and biotic vectors (animals), where seed dispersal has been identified as the most important process connecting communities (Figuerola and Green 2002; Soons *et al.* 2016). Nevertheless, pollen transfer also play an important role connecting communities (e.g., Harmon-Threatt *et al.* 2009; Schermer *et al.* 2018).

Movement of plants through "mobile linkers" (abiotic/biotic vectors) is essential for connecting communities enhancing gene and individual flow and therefore affecting biodiversity at different spatiotemporal scales (Jeltsch *et al.* 2013). A lot of species of flowering plants depend on biotic vectors including birds, mammals and insects (Ollerton *et al.* 2011). Among insects, bees are one of the most important group of pollinators worldwide (Dicks *et al.* 2016; IPBES 2016). Foraging patterns of pollinators in combination with flower phenology and reward traits (pollen and nectar) can be used to generate plant-pollinator networks to estimate plant and pollinator connectivity (Bosch *et al.* 2009). Natural and semi-natural habitats in fragmented and agricultural landscapes are important nesting and foraging sites for wild-bees that enhance diversity and assure pollination services for natural plant populations and agricultural croplands (Corbet 2000; Steffan-Dewenter *et al.* 2002; Westphal *et al.* 2003; Tscharntke and Brandl 2004).

1.1.5 Assessing connectivity in plants

How species move is mainly determined by the abiotic landscape and the ability to disperse (e.g., using mobile linkers) and essential for the connectivity between metacommunities (Nathan *et al.* 2008; Jeltsch *et al.* 2013). Connectivity can be defined in two types: structural and functional. Structural connectivity refers to the landscape configuration of habitat patches linked by spatial structures, (corridors, hedgerows, stepping stones); while functional connectivity explains the degree to which the landscape facilitates or impedes individual movement among suitable patches (Manel and Holderegger 2013). Functional connectivity, assumes that an organism can successfully move through the landscape and successfully reproduce in a new patch (Tischendorf and Fahrig 2000). It can be assessed by direct observation of how species move, or through genetic data (gene flow) among populations (Lowe and Allendorf 2010). Movement of organisms and their genes promotes gene flow constituting a source of genetic variation within populations maintaining effective population sizes, reducing the effects of genetic drift and extinction, enhancing biodiversity and the ecosystem functioning (Leimu *et al.* 2006; Staddon *et al.* 2010; Biggs *et al.* 2017; Liu *et al.* 2018; Wan *et al.* 2018).

In plants, due to the difficulty to track pollen or seeds, a promising option to analyze community dynamics estimating rates of migration and functional connectivity is through genetic data (gene flow) (Watkinson and Freckleton 2002; Lowe and Allendorf 2010). Molecular approaches are very diverse and are constantly being updated. To infer functional connectivity, molecular approaches use diverse markers displaying different amount of variation and modes of inheritance (dominant or codominant). Highly variable and codominant markers, such as microsatellites, provide reliable estimates of gene flow and dispersal in plants (Ouborg *et al.* 1999).

Microsatellites are simple sequence repeats, (SSR) widely distributed in the nuclear genome of eucaryotes of 2-6 bp in length (Bhargava and Fuentes 2010). They possess high polymorphisms ideal to study population dynamics of recent evolutionary events (Putman and Carbone 2014). Microsatellites are still widely used to answer many ecological questions and constitute a powerful tool for statistical genetic analyses used to infer connectivity patterns among populations (Selkoe and Toonen 2006).

1.2 Study system

1.2.1 Kettle holes as insular models to study metacommunities

In north of Germany up to 5% of arable land is occupied by a high density of natural small temporary wetlands (Brose 2001; Kalettka and Rudat 2006; Lischeid *et al.* 2017). These wetlands were formed by ice blocks due to retreating glaciers relicts from the Ice Age (Kalettka *et al.* 2001). They are commonly distributed in the north hemisphere and are known as "kettle holes" or "potholes" in Europe and North America respectively (Kalettka *et al.* 2001; Kalettka and Rudat 2006).

Kettle holes are very dynamic, they can experience severe wet-dry cycles or have a tendency to high water overflows (Kalettka and Rudat 2006). These characteristics contribute to surface water regulation and groundwater control of the landscape creating microclimate conditions establishing habitats with high biodiversity suitable for flora, fauna and endangered species (Gerke *et al.* 2010; Ungaro *et al.* 2014). Many studies highlighted the importance of kettle holes as hotspots of biodiversity, e.g. Oertli *et al.* 2002; Céréghino *et al.* 2012; Patzig *et al.* 2012; Platen *et al.* 2016.

These wetland habitats are good model systems to study ecological processes under the metacommunity framework. On the one hand, they provide suitable habitats for establishment of plant communities that can serve as shelter for animals, enhancing the biodiversity of the landscape at regional and local scales. On the other hand, for obligatory aquatic organisms, they are good models to study metapopulations and metacommunities because their condition as "aquatic islands" suitable for hygrophilous species that are surrounded by and embedded in unsuitable landscape matrix of crop fields (Brose 2001; De Meester *et al.* 2005). Therefore, these wetland habitats are good model systems to study ecological processes under the metacommunity framework. In addition, they can be regarded as metaecosystems where different interactions and movements might alter the spatial distribution of resources, acting as links coupling ecosystems (Gounand *et al.* 2018).

In agricultural landscapes, kettle holes provide ecosystems services, such as water regulation, species diversity, and cultural services and therefore are protected by federal law. Nevertheless, intensive land use practices still largely affect these important kettle holes habitat islands. Disturbances include structural degradation, severe pollution and habitat destruction (Céréghino *et al.* 2008), all factors threating the biodiversity (Kalettka *et al.* 2001), influencing organic matter turnover in surface sediments and edges (Kayler *et al.* 2017; Nitzsche *et al.* 2017), and decreasing the ecosystem services they provide. Therefore, a better understanding of ecological processes driving such relevant natural communities (for biodiversity conservation and as providers of ecosystem services) and their response to the environment is needed (Tscharntke *et al.* 2012) for an adequate management of these wetland habitats and the entire landscape.

1.2.2 Study area

For the three parts of this thesis, I used these kettle holes as ideal metaecosystems to evaluate different ecological processes under a metacommunity framework. Our study area is located in Brandenburg, Germany 100 km north of Berlin, in the so-called "AgroScapeLab Quillow" in the Quillow catchment, established by the Leibniz Centre for Agricultural Landscape Research (ZALF). This area comprises around 290 km^2 with intensive agriculture (65% of the land use) of maize, wheat and rapeseed and a high density of kettle holes (up to 2 per km^2) (Figure 1.1A).

We focused on different approaches, sampling different types of kettle holes for each study. To assess plant community assembly, a total of 46 kettle holes were selected and classified as "permanent" including Storage and Shore overflow types; and "ephemeral" or puddle types (see below) (Figure 1.1A). Ephemeral types are more dynamic and vulnerable than permanent ones. They can disappear completely in dry years and be easily ploughed and used as arable land (Kalettka and Rudat 2006). For these reasons, and due to the low amount of these kettle holes types in the landscape, only permanent kettle holes were selected for the other two studies. The selected four species for the second study were sampled in 20 kettle holes and for the third study we sampled wild bees in total 36 kettle holes.

The classification of the kettle holes for this thesis was modified from the one by Kalettka and Rudat (2006). These authors classified these wetland habitats based on hydrogeomorphic characteristics, where the most important variables were depth and slope, factors determining the amount of water that a kettle hole can retain. Based on this, these authors classified the kettle holes were ranged from permanent to temporarily flooded with three main types: storage type (S) (Figure 1.1B), shore overflow type (SO) (Figure 1.1C), and puddle type (P) (Figure 1.1D). The storage and shore overflow types are deep, relatively big with a permanent shore and constant influx of water; while the "non-permanent" or "puddle" type is small, flat and wadeable without a permanent shore and a dynamic water cycle (Kalettka and Rudat 2006). Based on these characteristics, for the first study we classified our sampling kettle holes in two groups: "flat ephemeral" (A) flat-sloped, less permanent and ploughed ones corresponding to puddle types, and "steep permanent" (B) steep-sloped and more permanent ones including storage and shore overflow types. For the second and third study, we sampled in "steep permanent" kettle holes only.

1.3 Aims and motivation

In highly intensive agricultural landscapes, connectivity between hotspots of biodiversity such as kettle holes, is very important to assure ecosystem functioning due to the services these wetland habitats provide. Plant species occurring in the kettle holes constitute as ideal systems to study processes of metacommunity assembly and connectivity.

The fundamental aim of this thesis was to infer the main ecological processes shaping plant communities and their response to the environment, from biodiversity patterns and key life-history traits involved in connectivity using ecological and genetic approaches under a

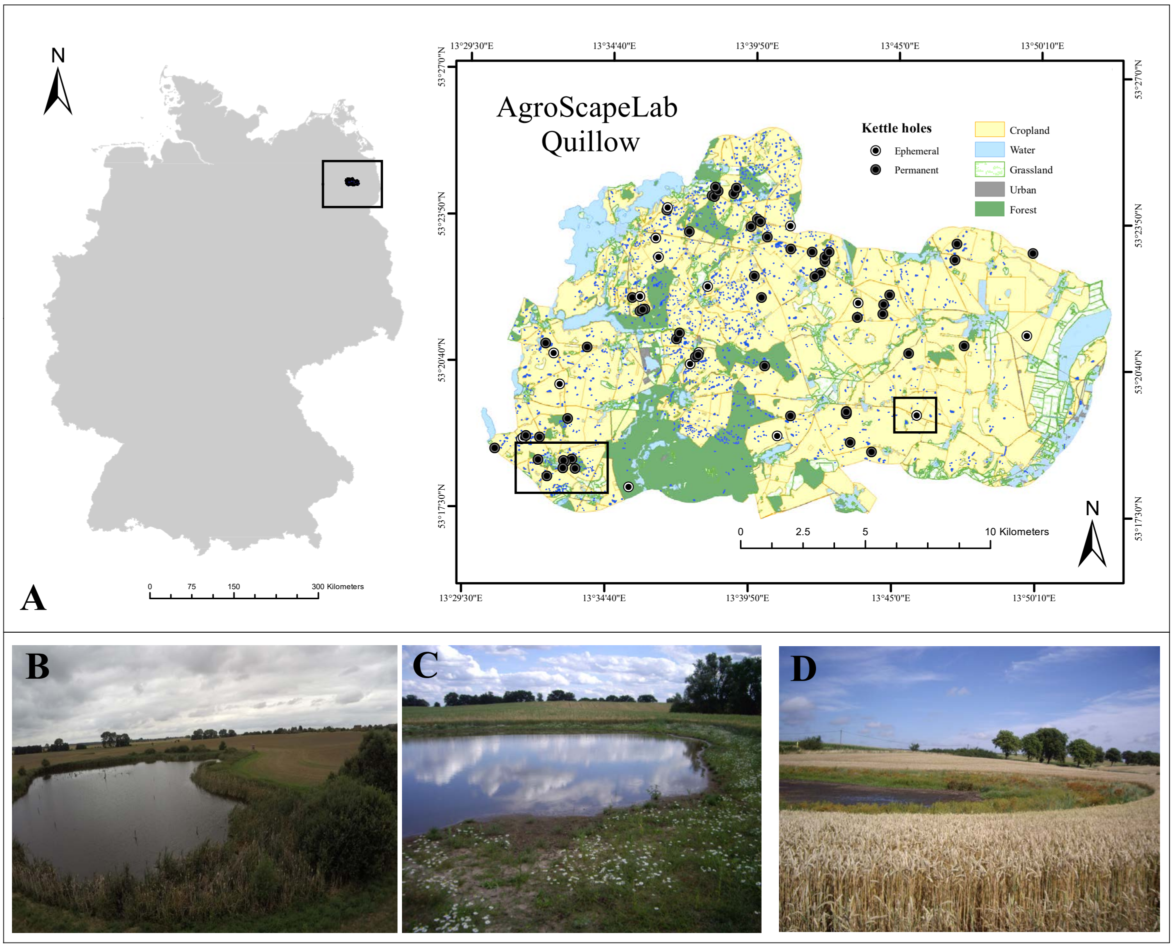


Fig. 1.1. Agricultural landscape in northeast Germany with high density of kettle holes. Upper part shows the study area (A). The so-called AgroScapeLabs located in the Quillow catchment is an area of around 290 km^2 with a high density of kettle holes shown in blue. Dots represent sampled kettle holes, plain black circles: Permanent; white border circles: Ephemeral. Permanent ponds are very deep and include S: Storage type (B) and SO: storage overflow types (C), while ephemeral ponds are flat and include P: puddle types (D). There is a lower occurrence of ephemeral kettle holes compared to permanent ones. Fotos: S. Lozada (B) and S. Stang (C, D).

metacommunity framework. Additionally, I evaluated wild-bee diversity patterns in relation to abiotic and biotic factors (i.e., habitat heterogeneity, flower resources). This provides first insights of the role of kettle holes harboring wild-bee species as mobile linkers connecting plant communities.

1.4 Structure of the thesis

This thesis is structured in five chapters. The current **first one** as a general introduction, the next three chapters (**Chapters 2-4**) correspond to three stand-alone scientific publications (published, under revision or in preparation) and lastly, a general discussion (**Chapter 5**).

In the **second chapter** (published in Ecology and Evolution), I evaluated the biodiversity patterns to infer ecological processes affecting plant communities' structure in two types of kettle holes. I focused on community assembly of plant communities in ephemeral vs. permanent ponds and their response to changing environments. I hypothesized that the type of kettle hole would be an important factor shaping the communities. Ephemeral and permanent kettle holes might act as environment filtering causing differences in habitat and therefore in plant composition. If so, following the metacommunity paradigms (Table 1.1), species sorting or mass effect process could be inferred in these heterogeneous types of kettle holes. I evaluated diversity patterns (alpha and beta) based on presence/absence data of all plant species occurring in the two types of kettle holes. In addition, I evaluated species richness in relationship with patch size and degree of isolation. Furthermore, I evaluated functional traits related to persistence, mating and dispersal of species occurring in the two types of kettle holes. Results showed differences in diversity and trait composition according type of kettle holes suggesting species sorting and niche differentiation processes; while mass effect processes were identified in ephemeral kettle holes only. A positive species richness-area was found in both types of kettle holes, but a negative relationship between species richness and isolation only for ephemeral ones. This suggests that flat ephemeral kettle holes strongly depend on seed dispersal and recruitment from a seed bank, whereas neighboring permanent kettle holes have a more stable local species diversity.

On the **third chapter** (under revision in Movement Ecology), I assessed the functional connectivity by means of molecular markers of four hygrophilous selected species: *Oenanthe aquatica*, *Lycopus europaeus*, *Typha latifolia* and *Phragmites australis* that differ in their dispersal modes and reproductive strategies. In *Oenanthe* and *Lycopus*, pollen transfer is mediated by insects and seed dispersal by water (occasionally endozochory by birds); while in *Typha* and *Phragmites* pollen and seeds are adapted to wind dispersal (occasionally exozochory by birds) (Hroudova *et al.* 1992; Kühn *et al.* 2004; Green *et al.* 2008; Kleyer *et al.* 2008; Brochet *et al.* 2009; Kleyheeg *et al.* 2015). All species can develop clonal rhizomes but is extremely rare in *Oenanthe aquatica* (Dickerman and Wetzel 1985; Kühn *et al.* 2004; Klimešová and Bello 2009; Packer *et al.* 2017) (Table 1.2; Figure 1.2). I evaluated the relative role of dispersal abilities compared with reproductive strategies of the selected species and the relationships between genetic diversity, genetic divergence (gene flow) with the landscape configuration. I argue that under a metacommunity framework, not only dispersal but also other life-history traits can determine the connectivity between local plant communities. I also evaluated the relationship between genetic diversity with patch size and degree of isolation. I hypothesized that traits related to reproduction and persistence, mainly clonal growth would have a significant effect on genetic diversity and genetic divergence; while mode of dispersal would play a minor role in gene flow. My results showed that the main factor affecting genetic diversity and genetic divergence is mating: ranging from outcrossing to selfing and clonality (degree of clonality). As expected, my results showed to have higher genetic diversity and gene flow in the non-clonal mainly outcrossing species and lower genetic diversity in the selfing and mainly clonal species. Clonal growth mainly explained gene divergence as well, although some results suggest a minor difference in gene flow between wind-dispersed species and insect-pollinated species. Additionally, my results showed an effect of environmental factors (plant richness, isolation) on the mainly outcrossing species only, probably related to increased competition and decreased patch availability for seedling establishment.

On the **fourth chapter** (in preparation for Landscape Ecology) I characterized the diversity of wild bee species occurring in the kettle holes as basis for understanding their role as pollinators connecting plant communities. I assessed alpha, beta, gamma diversity to evaluate biodiversity patterns of wild-bee species and the relationship with landscape heterogeneity, patch size and degree of isolation. I also characterized the community according related to body size (as an approximation of flight distance) as well as functional traits related to

TABLE 1.2: **Selection of plant species.** The four selected wetland species occurring in the kettle holes that differ in life-history traits regarding dispersal, persistence and mating.

TRAITS		***Typha latifolia***	***Phragmites australis***	***Oenanthe aquatica***	***Lycopus europaeus***
DISPERSAL	**Pollen**	Wind	Wind	Bees, flies, beetles	Bees, flies
	Seed	Wind, water, (birds)	Wind, water (birds)	Water, (birds)	Water, (birds)
PERSISTENCE	**Lifespan**	Perennial	Perennial	Annual. Perennial biannual	Perennial
	Clonal growth	Necessary	Necessary	None	Necessary
MATING	**Breeding systems**	Facultative xenogamous	Facultative xenogamous	Facultative autonomous	Facultative autonomous
	Self-compatibility	Self-compatible	Self-compatible	Self-compatible	Self-compatible

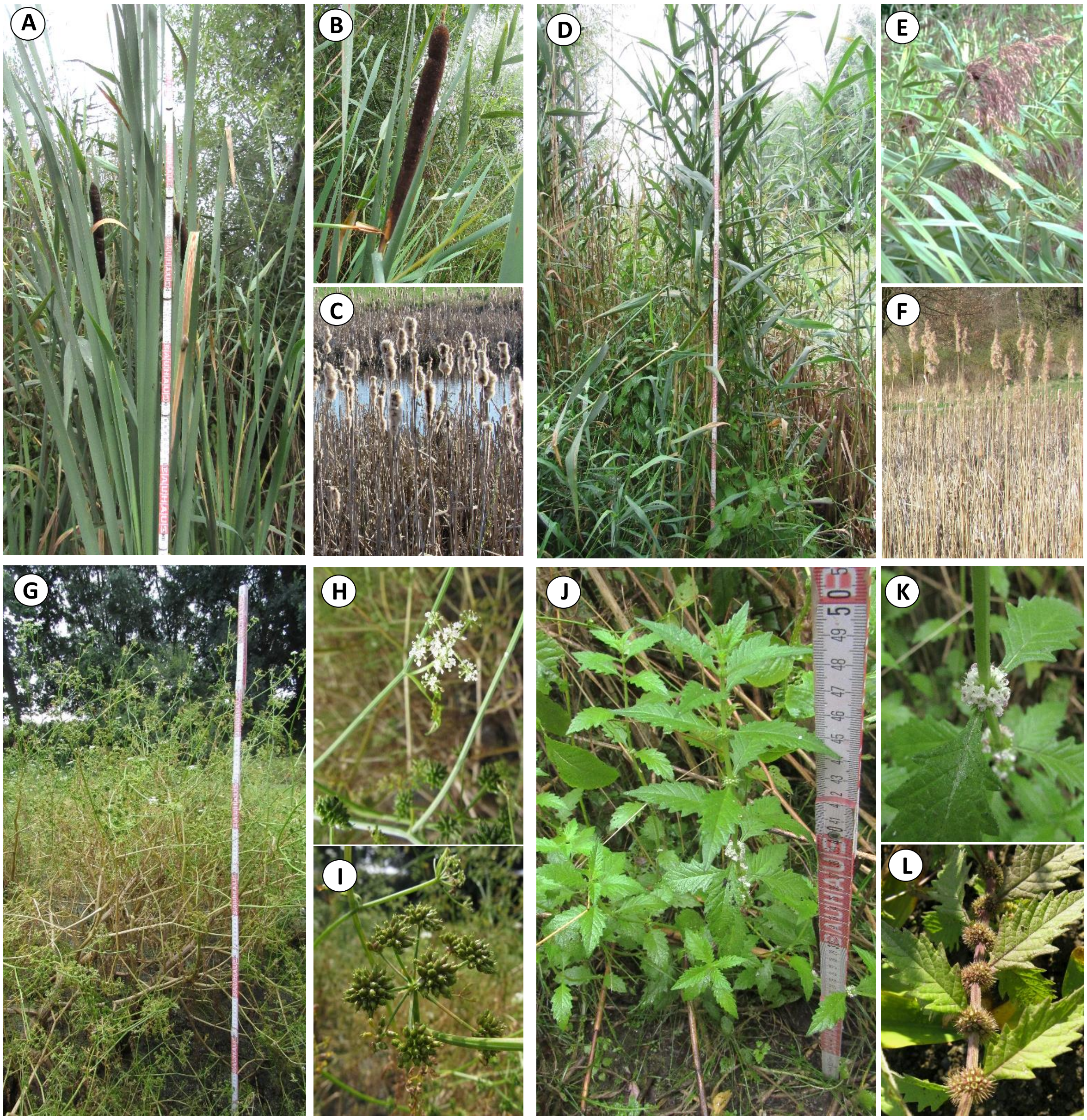


Fig. 1.2. Four hygrophilous selected species occurring in the kettle holes. *Typha latifolia* (A-C), *Phragmites australis* (D-F), *Oenanthe aquatica* (G-I) and *Lycopus europaeus* (J-L). *Typha* and *Phragmites* are wind-dispersed species with typical inflorescences for pollen transfer by wind (B, E), as well as infructescences (C, F). *Oenanthe* flowers (H) are pollinated by flies, beetles and bees and fruits (I) are mainly water-dispersed, or eventually by birds (exozoochory). *Lycopus* flowers (K) are pollinated by bees and fruits (L) are dispersed by water or endozoochory by birds. Fotos: S. Lozada Gobilard.

sociality, nectar foraging and nesting behavior to evaluate the importance of these wetland habitats as shelters. I assessed the effect of plant communities within the kettle holes on wild-bee diversity as well as the landscape heterogeneity at different distances surrounding the kettle hole. I hypothesized that habitat quality within kettle holes (i.e. flower resources) as well as habitat heterogeneity and patch size have a positive effect on bee diversity based on the assumption that different habitats provide a greater variety of nesting and food opportunities, while a negative effect of isolation degree was expected. My results showed a negative correlation between habitat heterogeneity and local wild-bee diversity at 100-300m buffer surrounding the kettle holes. No effect of patch size and degree of isolation on the entire bee community was found, but an effect of degree of isolation in large size social bumblebees only. This is the first contribution focusing on the diversity of wild-bees in the region highlighting the importance of these wetland habitats as refugees for pollinators of natural populations and croplands such as rapeseed (*Brassica repens*) as well as the effect of landscape factors on bee diversity.

My work is summarized in three independently readable research articles (**Chapter 2-4**). In the first paper (**Chapter 2**), I analyzed the data and lead the writing of the manuscript based on previous data collected by Susanne Stang. In second and third articles (**Chapter 3, 4**), I was the leading author, I was responsible for the study design, data analyses and manuscript writing. All articles contain suggestions and contributions from the all co-authors.

CHAPTER 2

Kettle holes act as environmental filters shaping plant communities

Permanent pond in Kraatz, Quillow catchment.

Title	Environmental filtering predicts plant-community trait distribution and diversity: Kettle holes as models of meta-community systems.
Authors	Sissi Lozada-Gobilard, Susanne Stang, Karin Pirhofer-Walzl, Thomas Kalettka, Thilo Heinken, Boris Schröder, Jana Eccard, Jasmin Joshi
Journal	Ecology and Evolution
Date of submission	Received 19 September 2018; Revised 22 November 2018; Accepted 7 December 2018; Published 21 January 2019.
Status	Published
Keywords	Plant diversity, species assembly, dispersal, life-history traits, seed bank, wetland vegetation, landscape diversity, biodiversity, disturbance.

2.1 Abstract

Meta-communities of habitat islands may be essential to maintain biodiversity in anthropogenic landscapes allowing rescue effects in local habitat patches. To understand the species-assembly mechanisms and dynamics of such ecosystems it is important to test how local plant-community diversity and composition is affected by spatial isolation and hence by dispersal limitation and local environmental conditions acting as filters for local species sorting. We used a system of 46 small wetlands (kettle holes) — natural small-scale freshwater habitats rarely considered in nature conservation policies — embedded in an intensively managed agricultural matrix in northern Germany. We compared two types of kettle holes with distinct topographies (flat-sloped, ephemeral, frequently ploughed kettle holes vs. steep-sloped, more permanent ones) and determined 254 vascular plant species within these ecosystems, as well as plant functional traits and nearest neighbor distances to other kettle holes. Differences in alpha and beta diversity between steep permanent compared with ephemeral flat kettle holes were mainly explained by species sorting and niche processes and mass effect processes in ephemeral flat kettle holes. The plant community-composition as well as the community trait-distribution in terms of life span, breeding system, dispersal ability, and longevity of seed banks significantly differed between the two habitat types. Flat ephemeral kettle holes held a higher percentage of non-perennial plants with a more persistent seed bank, less obligate outbreeders and more species with seed-dispersal abilities via animal vectors compared with steep-sloped, more permanent kettle holes that had a higher percentage of wind-dispersed species. In the flat kettle holes, plant-species richness was negatively correlated with the degree of isolation, whereas no such pattern was found for the permanent kettle holes. Synthesis: Environment act as filter shaping plant diversity (alpha and beta) and plant-community trait distribution between steep permanent compared with ephemeral flat kettle holes supporting species sorting and niche mechanisms as expected, but we identified a mass effect in ephemeral kettle holes only. Flat ephemeral kettle holes can be regarded as meta-ecosystems that strongly depend on seed dispersal and recruitment from a seed bank, whereas neighboring permanent kettle holes have a more stable local species diversity.

2.2 Introduction

A meta-community has been defined as "set of local communities that are linked by dispersal of multiple potentially interacting species" (Leibold *et al.* 2004). Local community assembly within a meta-community is therefore influenced by local interactions and regional processes (Wilson 1992; Logue *et al.* 2011). This interdependence of interactions and processes has been classified into four paradigms by Leibold *et al.* (2004) based on species characteristics (mainly dispersal), and environmental conditions: Species Sorting (SS), Mass Effects (ME), Patch Dynamics (PD) and Neutral Model (NM) (revised by Logue *et al.* 2011). In two of these processes, Species Sorting (SS) and Mass Effects (ME), environmental heterogeneity plays a role in filtering species due to niche difference (in case of SS) or due to a source-sink mechanism when patches are interconnected (in case of ME).

Environmental filtering is based on the idea that abiotic factors select species with particular traits and phenotypes to establish, persist and reproduce (environmental filtering sensu stricto), but establishment and persistence of species also depend on biotic interactions (Bartelt-Ryser *et al.* 2005; Kraft *et al.* 2015). Indeed, studies focusing on local–regional environmental gradients (Laliberte *et al.* 2014; Butterfield and Munson 2016) and (few) at global scale (e.g., Henriques-Silva *et al.* 2013; Le Bagousse-Pinguet *et al.* 2017) concluded that it is very difficult to separate biotic interactions from environmental filtering sensu stricto. In addition, identification of relevant environmental filters strongly depends on the selected scale (Münkemüller *et al.* 2014).

When organisms move under a meta-community framework, they connect habitats modifying the flow of resources and consequently the entire functioning of the ecosystem (Gounand *et al.* 2018). On this basis, the concept of "meta-ecosystem" was proposed by Loreau *et al.* (2003). These authors defined a meta-ecosystem as a set of ecosystems connected by spatial flows of energy, materials and organisms across ecosystem boundaries. In this meta-ecosystem dynamic, different types of movements or processes (e.g. predation, biomass recycling, mating aggregations) are involved in the coupling of ecosystems (Gounand *et al.* 2018), but empirical data on the movement ecology on relevant spatial scales for meta-community couplings is still limited.

In plant communities, passive movement has mainly been studied in seed dispersal (e.g., Figuerola and Green 2002; Soons *et al.* 2016) and less often through pollen despite its equal importance; for example for invasive species (Harmon-Threatt *et al.* 2009). Studies using genetic techniques to track both pollen and seed dispersal have successfully assessed functional connectivity of plant populations (Aavik *et al.* 2013) highlighting the importance of both dispersal processes. In addition, features such asexual reproduction (clonality), extreme longevity (trees, clonal plants), or the ability to survive under unfavourable conditions (seed bank) play an important role in connecting communities (Lienert 2004) allowing species to overcome disturbances and habitat degradation (Cain *et al.* 2000). In this sense, plant meta-communities can potentially form meta-ecosystems at a large scale. In the northern hemisphere, small water bodies formed by delayed melting of ice blocks of retreating glaciers, commonly called kettle holes or potholes (Kalettka *et al.* 2001; Tiner 2003; Kalettka and Rudat 2006), are ideal for studying meta-populations and meta-communities as they often form a network of aquatic and wetland 'island' habitats surrounded by an unsuitable matrix of intensively managed agricultural areas (Brose 2001; De Meester *et al.* 2005). These wetland ecosystems with their gradient in soil humidity support a high diversity of flora (e.g. Patzig *et al.* 2012) and fauna (Oertli *et al.* 2002; Gerke *et al.* 2010; Céréghino *et al.* 2012). However, intensive agricultural management threatens kettle holes causing structural degradation, eutrophication, pollution by plant-protection products, and direct habitat destruction (Kalettka *et al.* 2001; Céréghino *et al.* 2008; Altenfelder *et al.* 2014).

Given the high probability of disturbance and therefore potentially the highly dynamic nature of these small wetland ecosystems within the agricultural landscape, biotic connectivity patterns may strongly affect the species composition of the plant communities inhabiting these habitat islands (Cain *et al.* 2000; Bullock *et al.* 2002; Cottenie and De Meester 2004). In addition, different abiotic factors, especially hydrological and geomorphological characteristics (Brinson 1993; Kalettka and Rudat 2006) may act as local filters (Schmid *et al.* 2002) selecting for plant communities that may or not differ in plant diversity and functional traits in different types of kettle holes.

The aim of this study was to identify the main ecological processes driving plant diversity in meta-communities of two types of kettle holes: steep permanent and therefore less ploughed and less disturbed vs. flat, ephemeral, ploughable and more disturbed kettle holes, and their role as filters within an intensively managed agricultural matrix. To achieve this aim, we first compared plant diversity (alpha diversity) in relation with area of the pond (patch), and degree of isolation (number of ponds in the surroundings) to test whether larger areas harbour more species and whether more isolated patches harbour less species. Second, whether turnover of species and nestedness (beta diversity) differ in the two types of kettle holes for all plant species and including only wetland specialist species. Finally, we analyzed plant functional traits important for community dynamics including dispersal and movement abilities (pollen and seed dispersal) as well as colonization abilities (life span, seed longevity and self-compatibility systems) to test for niche differentiation processes (dissimilarities in traits) emphasizing on plant seed bank.

We hypothesized that the two types of kettle holes act as strong environmental filters shaping plant communities by different habitat conditions (Schmid *et al.* 2002). Under the meta-community paradigms, we hypothesized that two main ecological processes occur: species-sorting (SS) and mass-effect (ME) (Leibold *et al.* 2004). Similarity in species composition in both types of kettle holes and non-significant differences in dispersal abilities plus no effect of isolation would highlight the importance of SS, while a higher diversity in one of the type of kettle holes including all species of the other type might be an indication of source-sink mechanism related to a ME paradigm. A significant difference in trait distribution between communities would be an indication of niche differentiation between the two types of kettle holes.

2.3 Methods

2.3.1 Study area

Our study area was located in the "AgroScapeLab Quillow", an agricultural landscape laboratory in the Quillow river catchment area, which was established by the Leibniz Centre for Agricultural Landscape Research (ZALF) e.V. approx. 100 km North of Berlin (Germany, Brandenburg). This area comprises around 290 km^2 and contains a high density of small kettle holes (up to 2 per km^2) (Kalettka *et al.* 2005) connected by a shallow groundwater system (Kayler *et al.* 2017) and constantly influenced by seasonally changing hydrological conditions (Brose 2001; Kalettka and Rudat 2006; Figure 2.1). The water regime of the kettle holes from periodic to permanent in this region is influenced by a sub-humid climate with precipitation of 450–600 mm year^{-1} and potential evapotranspiration of 600–650 mm year^{-1} (Kalettka and Rudat 2006). The predominant land use of this area is intensive agriculture of maize, wheat, and rapeseed as the main crops.

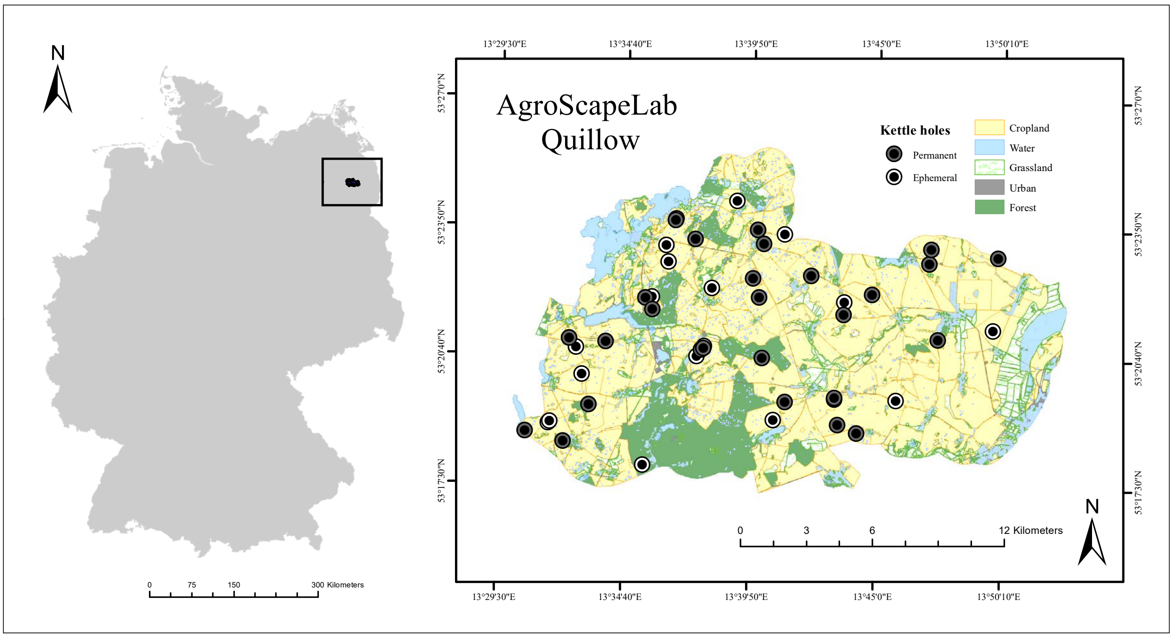


Fig. 2.1. Study area: the Agricultural Landscape Laboratory "AgroScapeLab Quillow" (www.bbib.org/experimental-platform.html) in the Quillow catchment area located in North-eastern Germany (Brandenburg). This agricultural landscape is characterized by a high density of kettle holes. Points denote our selected kettle holes (empty circles: flat/ ephemeral, filled circles: steep/ permanent). Percentage of land-use in the area is 65% cropland, 17% forest, 9% grassland, 5% water and 4% urban.

2.3.2 Selection and classification of kettle holes

The study area was divided into smaller sections where small kettle holes were visible from Google Earth satellite images from 2002. With random simulated numbers, we selected some kettle holes in each section trying to equilibrate the number of the distinct types (permanent and non-permanent). Some ephemeral kettle holes are very dynamic and can sporadically occur in the field in certain years depending on weather conditions (see below). If one of these dynamic kettle holes was not present during the initial sampling in the field, we selected another one close by if possible. We monitored plant-species composition of the kettle holes in July and August 2011.

Based on hydro- and geomorphological characteristics, Kalettka and Rudat (2006) proposed a classification key for kettle holes in North-East Germany. The first level of classification divides them in three groups: storage type, (S) shore overflow type (SO) and puddle type (P). The storage and shore overflow types (S and SO) are deep with a permanent shore and mostly periodically to permanently flooded; while the puddle (or non-permanent) type is flat without a permanent shore and mostly ephemerally flooded (Kalettka and Rudat 2006). In dry years, the puddle types can disappear completely and can be easily ploughed and used as arable land (Kalettka and Rudat 2006). Based on these characteristics and the vulnerability to agricultural practices, we classified our 46 sampling kettle holes in two groups: (A) flat-sloped, less permanent and ploughed ones corresponding to Puddle types, and (B) steep-sloped and more permanent ones including Storage and Shore Overflow types. For simplification, group (A) will be hereafter addressed as “flat ephemeral” and group (B) as “steep permanent” kettle holes.

2.3.3 Landscape parameters relevant for connectivity among wetland habitats

We calculated area and degree of isolation measured as the number of neighboring kettle holes within different radii: 20, 50, 100, 200, 500, 1000 and 2000 m using ArcGIS 10 (Esri 2011) based on land use and habitat type maps provided by Leibniz Centre for Agricultural Landscape Research (ZALF).

2.3.4 Plant identification and plant functional traits

We recorded presence or absence of all plant species occurring in the amphibian and terrestrial zone of the kettle hole. The amphibian zone is located between the open water body and (terrestrial) grassland vegetation next to the agricultural matrix (Pätzig *et al.* 2012). We identified the species according to Rothmaler (2011) excluding those that were cultivated in the arable matrix (e.g., *Zea mays, Hordeum vulgare, Brassica napus*). Three taxa – *Rosa*, *Rubus*, *Taraxacum* – could be determined to genus level only. For each species, Ellenberg indicator values (Ellenberg *et al.* 1991) were used to classify specialized wetland species (indicator value for moisture $\geq$ 7). The seed longevity index according to Bekker *et al.* (1998) - ranging from short-lived seeds = 0 to long lived = 1 - as well as data on species longevity were taken from the LEDA database (www.uni-oldenburg.de/en/landeco/research/leda/; Kleyer *et al.* 2008).

To test for functional differences in dispersal ability between plant communities occurring in permanent vs. ephemeral kettle holes, we analyzed the breeding system (selfing possible vs. non-selfers), the pollen vector (zoophily, anemophily and selfing), the dispersal syndrome (zoochory, anemochory, hydrochory, hemerochory and autochory) and life strategies for each plant species. The self-compatibility, pollen vector and life strategies dataset is based on BIOLFLOR (http://www2.ufz.de/biolflor/), the life span on the LEDA database (Kleyer *et al.* 2008) and the seed dispersal is mainly based on Rothmaler (2011) and completed with 3D Dispersal Diaspore Database (Hintze *et al.* 2013; www.seed-dispersal.info/terms-of-use.html) considering indices ranks > 0.5. All of the previously mentioned traits are in relation with colonization and dispersal abilities. We counted the total number of species that possess a particular trait and we calculated the percentage of species. Species can belong to more than one group, for example, to more than one dispersal syndrome (zoo-, anemo-, hemerochory). Those species were counted separately and summed up in the corresponding groups (see Supplementary material, Table A2 for details).

2.3.5 Plant seed bank

Soil samples were collected in April 2012 from 20 randomly chosen sites (ten permanent and ten ephemeral kettle holes; list in Supplementary material, Table A3). Soil samples were collected within the outer circumference of the kettle holes within the amphibian transition zone between open water body and grassland vegetation (ten random samples per site, ten cm deep, with a diameter of three cm) using a clean soil corer. Soil samples were stored in a cool dry place for three weeks until used for seed-bank assessment and soil-pH analysis. During three months, the number and identity of emerging seedlings of the soil seed-bank was weekly assessed in trays at the common garden site of the University of Potsdam using the seedling emergence method described in Kurtz and Heinken (2011).

For the seed-bank assays, seeds were divided into two wet treatments: flooded and non-flooded types to replicate natural conditions of permanent and ephemeral kettle holes. We tested whether germination varied according to treatment (flood, non-flood) and type of kettle holes (permanent, ephemeral). We measured the actual pH (soil/ 0.01 M calcium chloride solution ratio: 1:2.5) of the soil samples (using a WTW pH meter 325, Germany) to test whether putative differences in functional community composition are related to soil pH (see Ma *et al.* 2017).

2.3.6 Statistical analysis

We used GLMs (Generalized Linear Models) to test if the two different types of kettle holes differed in plant-species richness in relation to area and isolation degree. Due to overdispersion in the data, we explored two classes of models based on quasipoisson and negative binomial distribution. Since both models yielded similar results, we selected the quasipoisson model (Hoef and Boveng 2007) using the glm function in R. We tested if species richness of all plants or of specialized wetland plants only depends on kettle-hole area and if this effect differs between kettle hole types and whether the number of kettle holes in the surrounding has also an influence (isolation degree). For this, we previously tested which buffers (20, 50, 100, 200, 500, 1000 and 2000 m radii) influence plant-species richness and selected the

TABLE 2.1: Summary table of size (area), degree of isolation (number of neighbors within a 500-m radius) and total number of plant species found in the entire community and only the specialized wetland plants in both types of kettle holes: ephemeral and permanent.

		Permanent	**Ephemeral**	**Overall**
Area [m^2]	Mean ± SD	2228 ± 2127	1637 ± 1442	1997 ± 1893
	Min	290	240	240
	Max	8500	5600	8500
neighboring kettle holes	Mean ± SD	11.5 ± 7.4	11.7 ± 8.0	11.5 ± 7.5
	Min	0	0	0
	Max	28	26	28
Total species richness	Mean ± SD	49.3 ± 14.2	33.5 ± 13.6	43.2 ± 15.8
	Total	116	18	254
	Both	—	—	120
Wetland species richness	Mean ± SD	16.2 ± 7.0	12.4 ± 7.4	14.7 ± 7.3
	Total	28	6	80
	Both	—	—	46

minimum significant to fit the model. Due to very low number of ponds in small radii, we discarded the first three buffers (20, 50, 100m). A similar procedure was performed for the seed bank experiment, to test the influence of two factors: type of kettle hole and treatment (flooded or not) on germination.

To test the hypothesis that species composition varies between flat and steep kettle holes, first, we calculated overall beta diversity and its components: turnover and nestedness based on Jaccard dissimilarity matrices for presence-absence dataset with the function 'beta-multi' and three matrices containing the pairwise between-site values of each component of beta diversity with the function 'beta.pair' from the package betapart (Baselga and Orme 2012). Then we compared beta diversity between groups (types of kettle holes) using the function 'betadisper' based on permutation tests (PERMANOVA) under 95% confidence intervals around treatment centroids. Additionally, an overall beta diversity was calculated based on Ochiai index of similarity (Ochiai 1957). This index excludes double absences; it allows for chord or Hellinger transformation (Borcard *et al.* 2008) and proofed to be useful for plant communities (De Caceres *et al.* 2008). We computed an Ochiai index followed by a Hellinger transformation for our species presence-absence data. An ordination of Principal Coordinates Analysis (PCoA) was performed based on these Ochiai distances to visualize the plant communities.

Finally, to test whether percentage of plant species with a particular functional trait related to dispersal, reproduction or recruitment differ according type of kettle holes (permanent vs. ephemeral), we applied ANOVA tests because the data presented normality and homogeneity of variances (Supplementary material, Table A5).

2.4 Results

In total, 254 vascular plant species were identified in the 46 kettle holes studied (details in Supplementary material, Table A1, A2). Plant-species richness differed between the two kettle-hole types with a 41.5% lower species diversity in flat ephemeral kettle holes compared with steep, more permanent ones (138 vs. 236 species, respectively; $F_{1,44}$= 13.96, P<0.001). Of these, 120 plant species occurred in both habitat types, 116 exclusively in steep ones and 18 plant species exclusively in flat kettle holes (Table 2.1). In both habitat types, species richness increased with kettle hole area (Figure 2.2a). Increasing area was especially positively related to plant-species richness in permanent kettle holes when only specialized wetland plants were considered (Figure 2.2c). In contrast to the steep permanent sites, however, the

TABLE 2.2: Summary of statistical models used for landscape connectivity parameters (area and isolation) and for the seed-bank experiment in a subset of 20 kettle holes. Model selection was performed to explain the effect of size (area) and isolation degree (number of neighbors) on plant richness in both types of kettle holes in the entire community and for wetland species only; as well as the effect of types of kettle holes and wet treatment in germination from the seed bank. Due to over-dispersion, Generalized Linear Models (GLM) with a "quasipoisson" distribution were applied and model selection based on qAIC (lowest value) was performed (for details see Supplementary material, Table A4).

Best model	**Response variable**	**Predictors**	**Coefficient value**	**Statistic value**	**P-value**	**qAIC**
Seed bank experiment (n= 20)						
m01	Germination	Intercept	3.17	t = 20.62	P <0.001***	222.35
		Permanent	-0.63	t = -2.43	P <0.05*	
Landscape connectivity (n = 46)						
m12 (all species)	Species number	Intercept	3.44	t = 6.28	P <0.001***	167.47
		Log Area [m^2]	0.17	t = -0.88	P <0.001***	
		Neighbors 500m (a)	0.03	t = 0.85	P <0.001***	
		Permanent (b)	0.75	t = -0.24	P <0.01**	
		a:b	-0.03	t = -0.24	P <0.001***	
sp12 (wetland species)	Species number	Intercept	2.42	t = 9.94	P <0.001***	152.94
		Log Area [m^2]	0.26	t = 4.43	P <0.001***	
		Neighbors 500m (a)	0.04	t = 4.12	P <0.001***	
		Permanent (b)	0.91	t = 3.89	P <0.001***	
		a:b	-0.05	t = -3.67	P <0.001***	

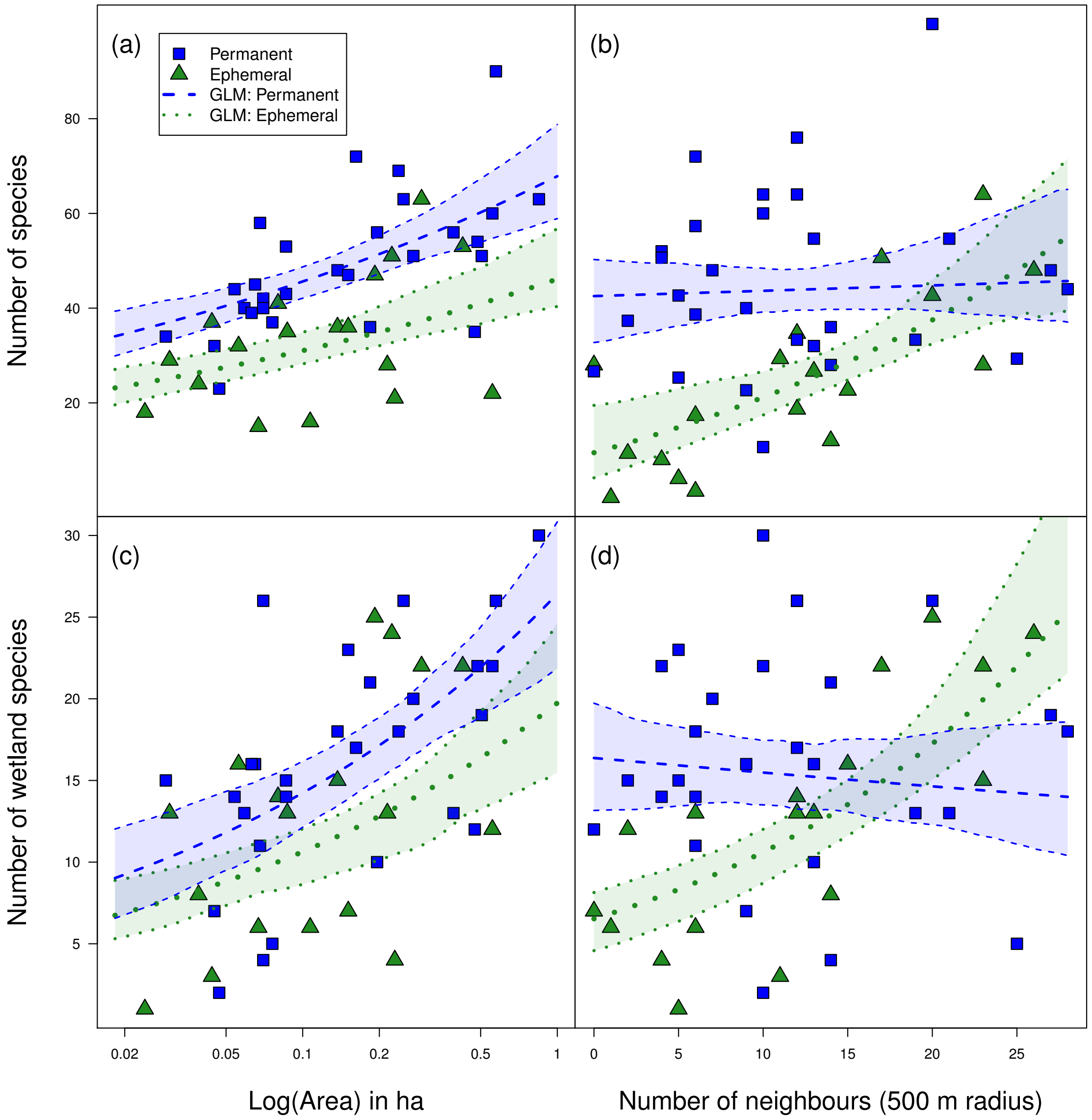


Fig. 2.2. (a) Relationship between plant-species richness and area (in ha) within the two types of kettle holes: ephemeral (flat) and permanent (steep); **(b)** number of neighboring ponds within a 500 m radius. There was a positive correlation between number of plant species and area in both types of kettle holes (all $P < 0.001$). In contrast, only species occurring within ephemeral ponds were positively influenced by the number of neighboring kettle holes within a 500-m radius (Number of neighbors x type of kettle hole $P < 0.001$). The same pattern was found when only wetland species were analyzed (**c:** all $P < 0.001$; **d:** Number of neighbors x type of kettle hole $P < 0.05$).

total plant-species richness as well as the number of wetland species was positively influenced by the number of neighboring ponds within a 500m-radius only in the flat ephemeral kettle holes (Table 2.2, Figure 2.2b, d). The best model explaining the relationship between species number (all and wetland species only) and area and number of neighboring kettle holes within a 500-m-radius was species number $\sim$ Area (ha) + Number of neighbors x* Kettle hole type (qAIC of 167.47 and 152.94 respectively; Table 2.2) (all models in Supplementary material, Table A4).

Soil pH in the seed-bank experiment showed a marginal but not significant difference between flat ephemeral vs. steep permanent kettle holes (7.1 ± 0.24 vs. 6.8 ± 0.44; $F_{1,419}$= 3.71 P = 0.069; Supplementary material, Figure A1). From a total of 34 different species that germinated, 19 species plus *Brassica napus* (Rapeseed of the surroundig matrix) could be identified to species level; no woody species were found (Species list in Supplementary material, Table A3). A total of 9981 seedlings germinated and seed abundance significantly varied between types of kettle holes ($F_{1,542}$= 5.48; P=0.01) with a higher seedling abundance in flat ephemeral than permanent kettle holes (22.3 ± 29.2 vs. 11.6 ± 15.3 respectively). Wet treatment (flooded vs. Non-flooded) had no effect in seedling abundance ($F_{1,542}$=1.14; P=0.29). The best fitted model was Germination $\sim$ Kettle hole type (qAIC = 222.35; Table 2.2).

High levels of beta diversity across study sites were found both in the entire community and for specialized wetland species (0.969 and 0.971 respectively) where species turnover (0.955 and 0.951) contributed considerably more to dissimilarity than nestedness (0.014 and 0.020; Table 2.3) in both communities. A Permutation Multivariate Analysis of Variances (PERMANOVA) showed a significant difference between the types of kettle holes for turnover of species and nestedness for the entire community (Turnover: $F_{1,44}$= 7.38; P<0.01; Nestedness: $F_{1,44}$ =10.19; P<0.01) and wetland community (Turnover: $F_{1,44}$= 11.44; P<0.01; Nestedness: $F_{1,44}$= 12.82; P<0.001). Overall beta diversity based on Jaccard similarity showed no difference between the types of kettle holes neither for the entire community, nor for the specialized wetland species ($F_{1,44}$= 2.11; P=0.15; $F_{1,44}$= 1.15; P=0.29). However, overall beta diversity based on Ochiai distances after a Hellinger transformation showed a separation in species composition between the two types of kettle holes (Figure 2.3a, b) when all plants species were considered ($F_{1,44}$= 4.37; P=0.04) and a tendency for separation when only wetland species were considered ($F_{1,44}$= 3.42; P=0.07) (Table 2.3).

TABLE 2.3: Species turnover, nestedness and overall beta diversity based on site dissimilarity (Jaccard dissimilarity) between the two types of kettle holes for the entire community and for the specialized wetland plants. Results of a Permanova (95% CI) show the comparison of the distance to centroids calculated according the type of kettle hole (permanent vs. ephemeral) for overall beta diversity and its components (turnover and nestedness) based on Jaccard dissimilarity. Overall beta diversity was also calculated based on Ochiai distances, which allowed for a Hellinger transformation for presence-absence data.

	Turnover (Jaccard)	**Nestedness (Jaccard)**	**Overall ß-diversity**	
			Jaccard distance	**Ochiai distance**
All species	0.955 (P<0.01**)	0.014 (P<0.01**)	0.969 (P=0.15)	P=0.04*
Ephemeral	0.872	0.051	0.923	
Permanent	0.933	0.017	0.951	
Wetland species	0.951 (P<0.01**)	0.020 (P<0.001***)	0.971 (P=0.29)	P=0.071
Ephemeral	0.837	0.089	0.927	
Permanent	0.924	0.028	0.952	

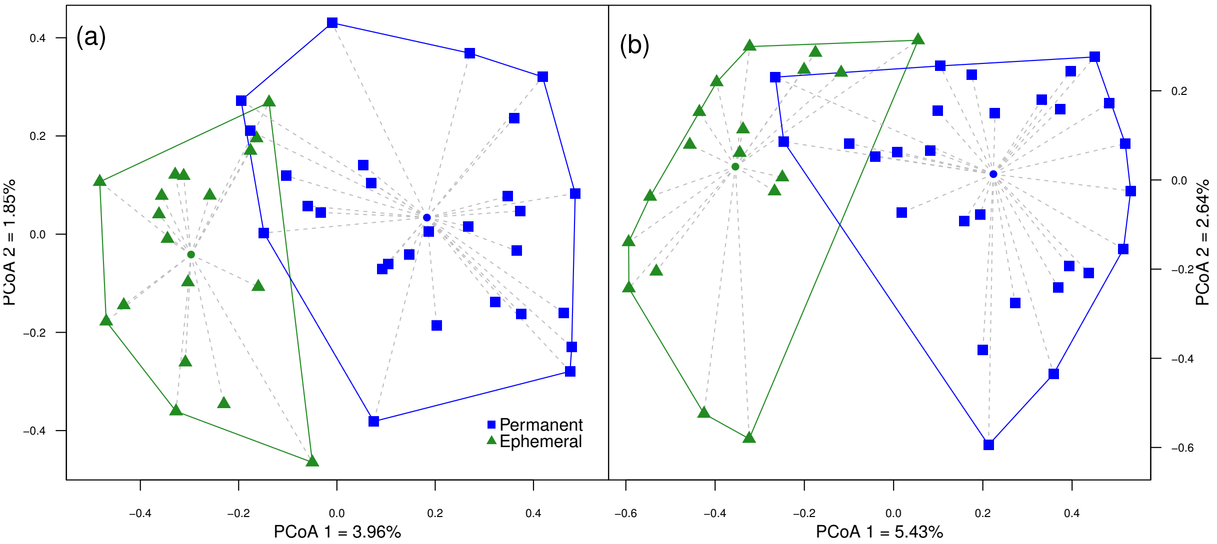


Fig. 2.3. Principal Coordinate Analysis using species composition of all **(a)** or specialized wetland plant species only **(b)**. An Ochiai matrix was generated as a standardization of data, following De Caceres *et al.* (2008) and afterwards a Hellinger transformation was applied. Results of PERMANOVA based on 99999 permutations showed a difference in plant-species composition according the kettle hole types for all species ($F_{1,44}$ = 4.37; P=0.04), and a tendency for difference when considering wetland species only ($F_{1,44}$ = 3.42; P=0.07).

Separation in plant-community composition between both types of kettle holes was reflected in the distribution of functional traits (Table 2.4). The majority of the species occurring in the ephemeral kettle holes had faster life cycles (higher percentage of annual and biennial plants; 64% ± 0.4 vs. 44% ± 0.5; $F_{1,32}$= 46.96; P < 0.0001; Figure 2.4b), and their seed bank was more persistent (0.5 ± 0.2 vs. 0.3 ± 0.2 ranging from short-lived = 0 to long lived seeds = 1; $F_{1,40}$= 91.31; P < 0.0001; Figure 2.4a). In addition, seed-dispersal abilities of the species varied according to the types of kettle holes with a slightly but significantly higher percentage of plants with zoochorous seed dispersal in ephemeral kettle holes than in permanent ones (76% ± 0.5 vs. 70% ± 0.4; $F_{1,38}$ = 10.79; P < 0.01). In contrast, fewer plant species relied on wind dispersal of seeds in ephemeral compared with permanent kettle holes (29% ± 0.4 vs. 38% ± 0.5; $F_{1,38}$= 10.79; P < 0.001; Figure 2.4d, e). The number of species that can produce seeds via selfing did not differ between the two types of kettle holes (all P > 0.1), but there was a slightly higher number of self-incompatible species (obligate outbreeders) in permanent kettle holes (28% ± 0.4 vs. 21% ± 0.4; $F_{1,43}$= 0.26; P < 0.0001). Moreover, in ephemeral kettle holes, we found a higher percentage of species that are mainly dispersed by humans (hemerochory) than in permanent ones (36% ± 0.4 vs. 26% ± 0.4; $F_{1,42}$= 0.26; P < 0.0001; Figure 2.4f). Finally, with respect to pollen vectors, there was a relatively lower percentage of insect-pollinated species in ephemeral kettle holes compared with permanent ones (59% ± 0.4 vs. 65% ± 0.4; $F_{1,38}$= 10.54; P < 0.01; Figure 2.4c).

TABLE 2.4: Comparison of plant traits affecting colonization and dispersal abilities within the two different types of kettle holes: flat ephemeral and steep more permanent. Data show percentage of species (% sp) plus Standard Deviation (SD). Note that the sum of species of both types exceed 100% as often one species possesses more than one trait (see Methods). Analysis of Variance (ANOVA) was performed to evaluate if the different functional traits differed according to type of kettle hole. Significance levels are indicated with asterisks: ***P<0.001, **P<0.01, *P<0.05..

Plant		**Ephemeral**		**Permanent**		**ANOVA**		
functional traits		**% sp**	**SD**	**% sp**	**SD**	**F**	**df**	**P**
Colonization abilities								
Self-compatibility	Self-compatible	80.7	0.23	80.7	0.31	0	1,43	0.995
	Self-incompatible	21.3	0.42	28.6	0.46	0.26	1,43	<0.001***
Recruitment	SLI1	0.541	0.27	0.371	0.29	91.31	1,40	<0.001***
Life span	Short-lived	63.7	0.43	43.8	0.49	46.96	1,32	<0.001***
	Long-lived	46.3	0.5	67.8	0.42	61.33	1,38	<0.001***
Dispersal abilities								
Pollen dispersal	Zoophily	59.4	0.48	65.2	0.46	10.54	1,38	0.002**
	Anemophily	37	0.49	36.4	0.48	0.07	1,38	0.7
	Hydrophily	1.3	0.06	4.1	0.17	11.58	1,38	0.002**
	Selfing	56.9	0.49	55.4	0.49	1.69	1,38	0.2
Seed dispersal	Zoochory	76.3	0.41	69.7	0.45	10.79	1,38	0.002**
	Anemochory	28.9	0.45	37.6	0.48	23.21	1,38	<0.001***
	Hydrochory	45.6	0.49	46.3	0.49	0.07	1,38	0.8
	Hemerochory	36.3	0.48	26.3	0.43	16.58	1,42	<0.001***
	Autochory	10.3	0.27	17.8	0.38	20.7	1,38	<0.001***

2.5 Discussion

The aim of this study was to identify the main ecological processes driving plant diversity in two types of kettle holes — steep permanent vs. flat ephemeral —within an intensively used agricultural landscape under a meta-community framework. We compared plant features such as life span, seed dispersal ability, pollen transfer, seed bank and seed longevity in these two wetland types. Our results suggest that the type of kettle holes acts as a strong environmental filter for plant communities, but this system cannot be explained by one meta-community paradigm only. Whereas flat ephemeral kettle holes can be regarded as meta-communities that strongly depend on seed dispersal and recruitment from a seed bank, the plant species-richness of neighboring permanent kettle holes was not influenced by degree of isolation and had a more stable local species diversity. Furthermore, the significant difference in trait distribution between communities is an indication of niche differentiation between the two types of kettle holes. Hence, plant functional traits offer good insights in understanding the role of local environmental conditions (local filters) and regional species sorting in these freshwater islands within an intensively managed agricultural matrix.

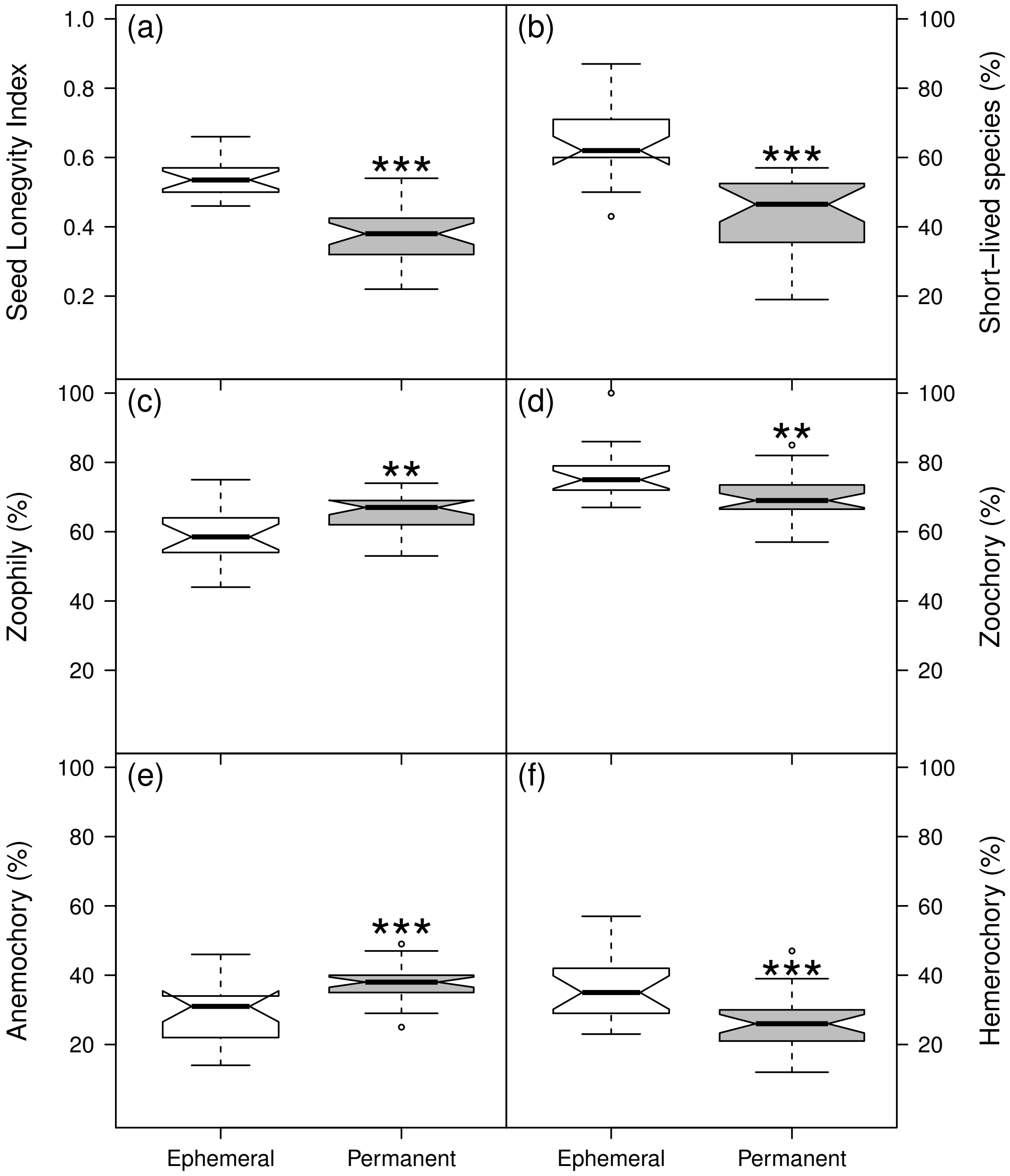


Fig. 2.4. Plant traits important for colonization: seed longevity **(a)** and individual life span **(b)**. The seed-bank longevity index (ranging from short-lived = 0 to long lived seeds = 1) was significantly higher in the ephemeral kettle holes (P<0.001) harbouring more persistent seeds. In contrast, in these ephemeral, flat and more disturbed kettle holes, more short-lived plants (non-perennials) with a faster life cycle (annuals, biannual) were found (P<0.001). In addition, plant traits for pollen movement **(c)** and seed dispersal **(d-f)** differed among types of kettle holes (all P<0.01). Permanent kettle holes harboured a higher percentage of species pollinated by animals and seeds dispersed by wind (all P<0.01); and ephemeral kettle holes contained more species with seeds dispersed by animals and human related vectors (all P<0.01).

2.5.1 Species sorting and mass effect processes at different scales

Apart from the rare and endangered 21 plant species present in the state red-list of Brandenburg (Rote *et al.* 2006), the overall considerable diversity of 254 plant species found in 46 small kettle holes within the matrix of intensively managed agricultural fields, substantially enhances biodiversity at the landscape scale. As expected, in both types of kettle holes, we found a positive correlation between species richness and habitat size where a larger area harbours a higher number of species, as it was previously well documented for small wetland habitats (e.g., Jeffries 2012 and references therein). This can be generally explained by the framework of "environmental heterogeneity" where a wider range of habitats is suitable for more different plant species in larger habitats (Stein *et al.* 2014). However, this relationship can vary among taxa (Oertli *et al.* 2002) and diversity is not always reflected by species richness but by the diversity of functional traits. For example, a previous study in the same region by Pätzig *et al.* (2012) found no clear pattern regarding macrophyte species richness.

Differences in alpha and beta diversity between types of kettle holes without a change between the entire community and the wetland-plant community suggest that different environmental conditions act as local filters (Schmid *et al.* 2002) driving functional niche occupancy (Li *et al.* 2018) reflected in different plant functional traits (Figure 2.3, 2.4). Under the framework of meta-community paradigms when habitat patches are environmentally heterogeneous, Species Sorting (SS) or Mass Effect (ME) processes may occur (Leibold *et al.* 2004). Different environmental conditions of the kettle holes provide a different habitat quality that in combination with different dispersal strategies affect community composition supporting the Species-Sorting process (Leibold *et al.* 2004) at a regional level. In concordance, our beta diversity results show that plant communities between kettle holes are mainly explained by species turnover (species replacement from one pond to another) without differences between the entire and the wetland community (Table 2.3). Similar results were previously reported for meta-communities of aquatic plants and macroinvertebrates (Viana *et al.* 2016; Hill *et al.* 2017), supporting the Species Sorting process at a regional scale.

A low number of unique species in ephemeral kettle holes (18 out of 254 species), but not in permanent kettle holes (116/254) suggests a Mass Effect process, where permanent kettle holes might be acting as a source and ephemeral ones as sink supported by the high number of shared seedlings that germinated in both types of ponds (21/34; Table 2.1). A negative relationship with distance to neighboring ponds in flat kettle holes (Figure 2.2d) suggests that spatial colonization (dispersal filtering) is also an important process driving community assembly in these ephemeral habitats. In addition, turnover of species and nestedness differed depending on type of kettle holes with a higher turnover in permanent and a higher nestedness in ephemeral kettle holes (Table 2.3). These results show that the larger permanent ponds also follow the SS paradigm harbouring species with a higher replacement than ephemeral ones. The higher nestedness in ephemeral ponds suggests that they are a subset of the species assemblage of the permanent ponds supporting the Mass Effect process at a local scale.

Finally, if we only consider ephemeral kettle holes and assume that patches among them are similar, the dynamic state of these kettle holes (drying and reappearing) might reflect a patch dynamic paradigm were patches can be occupied or unoccupied where local diversity is limited by dispersal (Leibold *et al.* 2004). It is known that temporal variation in patch suitability and availability in combination with spatial colonization and founder effects play an important role shaping communities (Jeffries 2008; Mahaut *et al.* 2018). In our system, ephemeral kettle holes possessed a more persistent seed-bank source of propagules (Figure 2.4a) in combination with short-lived species (Figure 2.4b) suggesting that the species' life cycles are more in synchrony with patch availability enabling persistence on the sites over periods when the ephemeral kettle holes are not present (e.g. Alderton *et al.* 2017; Poschlod and Rosbakh 2018). Even though we found a low number of competitive species in both ephemeral and permanent ponds (20% and 30%) (data not shown), the relationship between migration (dispersal) and local dominance and colonization–competition trade-offs are fundamental to assess patch dynamics (Logue *et al.* 2011). Since our data (presence-absence) lack abundance information, further experiments are needed to confirm these hypotheses.

2.5.2 Linking species sorting with movement ecology

In plants, it is mainly seed dispersal that defines movement ecology (Nathan *et al.* 2008) and therefore the most important factors influencing seed movement are dispersal vectors (biotic and abiotic) in combination with motion abilities; followed by environmental filters (Damschen *et al.* 2008). Both, environmental conditions and spatial distribution of suitable habitats can lead to environmental and dispersal filtering (seed arrival, recolonization events) and both are shaping local species communities (Fraaije *et al.* 2015). Additionally, it has been shown that pollen transfer is as an important limiting factor connecting populations with consequences in biodiversity and regeneration (Schermer *et al.* 2018) or economic loss in agricultural landscapes related to invasive weeds (e.g. Fénart *et al.* 2007). Our results showed a higher number of zoophilous plant species (insects as pollen vectors) in permanent kettle holes. These results suggest that permanent kettle holes provide habitat and food source to harbour a higher number of pollinators (e.g. wild bees and bumblebees), whose community might be related to higher plant diversity and habitat heterogeneity found in the permanent kettle holes compared with the ephemeral ones. This might be related with the higher number of obligate outbreeders (self-incompatible) species found in these permanent kettle holes (Supplementary material, Figure A2).

Our results showed a difference in dispersal syndrome depending on environment where biotic dispersal vectors (zoo-, hemerochory) seemed to be more effective in ephemeral kettle holes and abiotic vectors (anemochory) in permanent kettle holes (Figure 2.4d-f). A possible explanation to these results might be that kettle holes offer a different accessibility for seed dispersers, mainly biotic, and a different degree of exposure and vulnerability to intensive land use. Even though both types of kettle holes constitute a source of food and water for animals (deer, wild boars, migratory birds), which might disperse the seeds while foraging (e.g. Figuerola *et al.* 2003; Dovrat *et al.* 2012; Soons *et al.* 2016; Flaherty *et al.* 2018), permanent kettle holes harbour a significantly higher number of long-lived (Supplementary material,

Figure A2) and tall plant species that might offer a better shelter for animals, or form less accessible dense thickets compared with ephemeral kettle holes. Consequently, ephemeral kettle holes are in more direct contact with the intensive land-use surroundings and farming activities (e.g. tractors for harvest), which could easily act as potential —hemerochorous — seed dispersers (Figure 2.4f).

2.5.3 Ephemeral kettle holes as stepping stones to conserve plant diversity

The dynamic state of ephemeral kettle holes provides different environmental conditions for colonization events and different dispersal vectors (highly mobile birds or humans via agricultural machinery) compared with permanent kettle hole ecosystems consisting of more long-lived plants. Both types of ponds form a dense network of freshwater island habitats where ephemeral ponds might act as stepping-stones due to the common, unique and high turnover of species enhancing the overall plant diversity at the landscape scale. The importance of ephemeral kettle-hole density for the maintenance of plant-species richness is supported by a low weed diversity recently found in agricultural fields within the same area (Müller-Nilsson 2018) suggesting a low permeability for wild plants of the agricultural matrix surrounding the ephemeral kettle holes.

A previous study in the region suggested that management and conservation policies should consider all types of kettle holes (Pätzig *et al.* 2012). Other studies focused on temporary flooded depressions provide measures to conserve plant communities based on management of water-level fluctuations and land-use practices (Altenfelder *et al.* 2016a, b). We highlight the importance of flat ephemeral kettle holes as key habitats acting as stepping stones to preserve plant diversity within this agricultural landscape (Hallmann *et al.* 2017). Despite their biodiversity and the ecosystem services these small water bodies provide, conservation policies are not well established yet, excluding them from freshwater science and international nature conservation policies (Biggs *et al.* 2017). To overcome this problem, Hill *et al.* (2018) recently proposed practical steps to focus on "pondscapes" and their impact on society. Our study contributes to a better understanding of these ponds but long-term studies to understand the dynamics of these meta-communities are needed (Ruhí *et al.* 2017) for a future integration of these pondscapes into policies and a sustainable management of these agricultural landscapes.

2.6 Conclusion

Our study shows that differences in alpha and beta diversity between steep permanent compared with ephemeral flat kettle holes are mainly explained by species sorting and niche processes at regional scale, while mass effect and dispersal limitation processes are detectable at local scale in ephemeral kettle holes only. We highlight the importance of supporting a high density of flat ephemeral kettle holes within intensively managed agricultural landscapes to sustain population dynamics and plant diversity. Flat ephemeral kettle holes are more vulnerable to environmental filtering particularly related to human activities compared with steep-permanent kettle holes. We suggest to establish management and conservation policies focusing on these freshwater bodies considering their function as stepping stones enhancing plant-diversity in intensively used agroecosystems.

2.7 Acknowledgements

We would like to thank Christine Kurtz for contribution to recording of the species list in the field and Rachael Okong'o for soil pH measurements and assessment of soil-seed bank. We are very grateful to the anonymous reviewers whose comments and suggestions improved the original version of the manuscript. We acknowledge the support of Deutsche Forschungsgemeinschaft (DFG) and Open Access Publication Fund of Potsdam University. KPW acknowledges funding from BiodivERsA/FACCE-BMBF for the project BASIL.

2.8 Funding

This work was supported by Deutsche Forschungsgemeinschaft (DFG), in the framework of the BioMove Research training group (DFG-GRK 2118/1), Open Access Publication Fund and BiodivERsA/FACCE-BMBF, BASIL project.

2.9 Authors contribution

JJ, SS, BS, TK, JE and TH designed the study. SS and KPW collected the data in the field. SS, SLG and BS analyzed the data, SLG, JJ, KPW, TK, TH, BS, and JE wrote the paper, contributed critically to the drafts and gave final approval for publication.

2.10 Data Accessibility

Data is available in Supplementary Materials and raw data is deposited in the ZALF Repository http://www.doi.org/10.4228/ZALF.DK.102.

2.11 Supplementary Material

Table A1: Characteristics of the kettle holes.
Table A2: Characteristics of the plant species including colonization and dispersal traits.
Table A3: List of species found in the seedbank in a subset of 20 kettle holes.
Table A4: Detailed Generalized Linear Models with a quasi-poisson distribution selection based on Explanatory Deviance and qAIC.
Table A5: Best fitted Linear Models and ANOVAs of colonization and dispersal traits.
Fig. A1: Soil analysis of a subset of 20 kettle holes.
Fig. A2: Additional colonization and dispersal plant traits.

CHAPTER 3

Genetic diversity and connectivity depend on the degree of clonality

Seeds of Typha latifolia close to Buchenhain, Uckermark.

Title	Genetic diversity and connectivity in wetland plant metapopulations depend on the degree of clonality
Authors	Sissi Lozada-Gobilard, Christian Schwarzer, Rodney Dyer, Ralph Tiedemann and Jasmin Joshi.
Journal	Movement Ecology
Date of submission	Submitted 30th April 2019
Status	Under review MOVE-D-19-00035
Keywords	*Lycopus europaeus, Oenanthe aquatica, Phragmites australis, Typha latifolia,* dispersal, breeding systems, clonal growth, metapopulations, genetic diversity, genetic divergence, kettle holes, wetlands

3.1 Abstract

Background: Under the meta-community framework, dispersal between local communities is essential for the entire functioning of an ecosystem. In plant meta-communities, long-distance dispersal is both attenuated and directed by specific movement vectors including animals, wind and/or water. While much is known about the role of dispersal on genetic diversity and genetic divergence or connectivity, its relative importance compared to other life-history traits such as clonal growth and breeding system (i.e., selfing vs. outcrossing) is not well understood. **Methods:** In our study, we evaluated the relationship between dispersal mechanisms, modes of reproduction (degree of clonality) and breeding system as well as of landscape factors such as patch size and degree of isolation on genetic diversity and genetic divergence in a metacommunity system. In an insular system of kettle holes within an intensive agricultural landscape, we studied four widespread wetland plant-species that differ in their dispersal mechanisms and reproductive strategies. We used neutral genetic markers (microsatellites) to calculate genetic diversity and genetic divergence to infer breeding systems and gene flow in these species. **Results:** Our results showed that reproduction systems, from outcrossing to selfing and predominantly clonal reproduction, are the main factors shaping genetic diversity and genetic divergence in metapopulations of wetland species, as exemplified in our island habitats within an intensive agricultural matrix. In addition, dispersal mechanisms mattered for gene flow and connectivity: There was a higher gene flow respectively a lower genetic differentiation among populations in wind vs. insect pollinated species in this insular system. Genetic diversity, however, was mainly explained by clonal growth and breeding system. The outcrossing insect-pollinated and rarely clonal species *Oenanthe aquatica* exhibited the highest genetic diversity and gametic richness in comparison to the other three more clonal species. **Conclusions:** The main factor affecting genetic diversity and genetic divergence in metapopulations of the wetland species investigated was the breeding system (ranging from outcrossing to selfing and clonality); dispersal mechanisms played a minor role for diversity, but were related to gene flow and connectivity. Our findings are important for a functional understanding of plant metacommunities and may provide relevant information for an appropriate implementation of conservation policies.

3.2 Background

Movement of organisms is essential to assure connectivity in fragmented landscapes affecting individuals, populations, communities and ecosystems at both short-term ecological and long-term evolutionary time-scales (Nathan *et al.* 2008). Lack of connectivity can cause a decrease in the probability of population persistence, a decrease in individual fitness and genetic diversity within populations, and may lead to increased genetic differentiation among populations (e.g., Leimu *et al.* 2006). Many studies have shown that increasing habitat connectivity with corridors or stepping stones has a positive effect on population persistence in fragmented landscapes. For example, habitat connectivity can reduce extinction risk (Gonzalez and Chaneton 2002; Yang *et al.* 2016), enhance species diversity and gene flow (Hill *et al.* 2016; Wan *et al.* 2018) and contribute to ecosystem functioning (Staddon *et al.* 2010; Liu *et al.* 2018).

Landscape configuration determines the movement of organisms and therefore the degree of connectivity of suitable habitat patches (Taylor *et al.* 1993). The concept of structural connectivity refers to the distribution of habitat patches, while functional connectivity explains the degree to which the landscape facilitates or impedes individual movement among suitable patches (Manel and Holderegger 2013). The interaction between landscape structure and realized movement is generally considered to be a species-specific trait (Tischendorf and Fahrig 2000). Functional connectivity can be assessed by direct observation or through genetic techniques (Lowe and Allendorf 2010), the latter assuming that the successful movement of an organism through the landscape results in successful reproduction in a new patch (Tischendorf and Fahrig 2000).

Plants exhibit a passive mode of dispersal due to their sessile nature. Hence, dispersal is often mediated through external vectors such as animals, wind or water (Aavik *et al.* 2014). In this sense, plant functional connectivity has been defined as "the effective dispersal of propagules or pollen among habitat patches in a landscape" (Auffret *et al.* 2017). Seed dispersal is known to be the most important process connecting plant populations (Figuerola and Green 2002; Soons *et al.* 2016), but many recent studies highlight the importance of pollen transfer as well (e.g., Harmon-Threatt *et al.* 2009; Schermer *et al.* 2018). In addition to the modes by which propagules are dispersed, other external factors such as resource availability, disturbance, response to environmental change, pathogen/herbivore attack, or competition between plants, are also important for plant-species movement and may attenuate functional connectivity (Auffret *et al.* 2017). Especially in fragmented landscapes, life-history traits such as asexual reproduction (clonality), extreme longevity (trees, clonal plants), or the ability to survive under unfavourable conditions (seed bank) play an important role for population viability (Lienert 2004), enhancing species' resilience to disturbances, habitat degradation (Cain *et al.* 2000) and climate change (Graae *et al.* 2017).

One important but usually neglected trait in plant meta-community functioning is clonal growth. Clonal growth is widely distributed in all biomes, present in 51% of angiosperms from temperate regions, and particularly common in wetland habitats (Klimešová *et al.* 2012, 2018). Many studies have demonstrated the ecological importance of clonal growth in plant competition, the ability to deal with disturbances (resilience), nutrient acquisition and reproduction (Bazzaz 1996; Stueffer *et al.* 1996; Klimešová *et al.* 2012, 2017, 2018). A horizontal rooting stem is the most common mode of clonal growth (Klimešová and Klimeš 2008). The ability to grow horizontally provides species with a key function of persistence on a spot associated with a higher probability for plants to remain in a suitable patch (Graae *et al.* 2017). In an insular system, there is a higher risk of moving into an unhospitable matrix. Under such circumstances, clonal reproduction should be advantageous. However, long-term persistence in combination with a lack of sexual reproduction (selfing and restricted gene flow) might cause loss of genetic diversity and mutational meltdown, ultimately leading to population decline or extinction (Jiménez-Alfaro *et al.* 2016; Roberts *et al.* 2017), but see Ally *et al.* (2010).

Even though there are many studies on how dispersal mechanisms affect functioning of metacommunities, little is known about the role of reproductive systems related to dispersal, or more specifically, the amount and importance of dispersal in clonal plants in a meta-community context. Clonality can determine on-spot persistence, providing an effective strategy to cope with changing environmental conditions resprouting after damage (Ottaviani *et al.* 2017; Klimešová *et al.* 2018). Therefore, the degree of clonal reproduction vs. sexual reproduction involving gene flow by pollen and seeds may affect species distribution and diversity at local and meta-community levels, as well as spatial patterns of genetic diversity and population differentiation.

In the present study, we aimed to identify how reproductive systems in combination with dispersal mechanisms shape genetic diversity and connectivity in an insular system. Specifically, we correlated genetic patterns with the mode of reproduction (outcrossing, selfing, clonality) and dispersal related factors such as wind direction, wind speed, and availability of pollinators, as well as landscape factors such as patch size and degree of isolation. We expected that both dispersion vectors and modes of reproduction will affect genetic diversity and genetic divergence. On the one hand, we hypothesized that an outcrossing species (even if it is also able to reproduce clonally) will depend on the transport of pollen and dispersal of seeds between populations, which will cause low population differentiation (F_{ST}), few genetically distinct clusters) and high population genetic diversity (H_E, H_O) and gametic richness (R) with a low level of linkage disequilibrium r_d and no excess (or deficit) of heterozygotes (F_{IS}). On the other hand, in self-compatible and clonal species, dispersal vectors are expected not to play an essential role in gene movement, causing higher populations differentiation and a lower within-population genetic diversity due to the longevity of the once established clones (Table 3.1).

We used a meta-ecosystem of island-like aquatic habitats called kettle holes, whose spatial configuration on the landscape make them ideal to study meta-populations and meta-communities (Tiner 2003; Kalettka and Rudat 2006; Lozada-Gobilard *et al.* 2019). These small water bodies, remnants of the last glaciation, mainly occur in the northern hemisphere. For aquatic and wetland organisms, they form a distributed network of habitats surrounded

TABLE 3.1: **Framework of hypotheses.** Predicted hypotheses covering the relationship among breeding systems, dispersal, connectivity and genetic diversity. Plus (+) and minus (-) symbols in "Dispersal" indicate the probability of pollen or seed to be transported between populations. Connectivity is explained by the degree of genetic divergence: the better connected the populations the lower the Fixation Index (F_{ST}) and the fewer genetic clusters. In outcrossing species, genetic diversity (H_E, H_O) and gametic richness (R) are high and associated with F_{IS} (Inbreeding coefficient) and rd (Linkage Disequilibrium) close to 0, while in selfing and clonal species genetic diversity parameters decrease and F_{IS} and r_d increase.

BREEDING SYSTEMS	**DISPERSAL**		**CONNECTIVITY**		**GENETIC DIVERSITY**			
(clonality degree)	**Pollen**	**Seed**	F_{ST}	**No. genetic clusters**	H_E, H_O	**R**	F_{IS}	r_d
OUTCROSSING **(Non-clonal)**	$+$	$+$	Small	Small	High	~ 1	~ 0	~ 0
SELFING **(clonal possible)**	$-$	$+$	Intermediate	Intermediate	Medium	< 1	> 0	> 0
CLONAL **(mainly/only clonal)**	$-$	$-$	Large	Large	Low	~ 0	not clear	> 0

by an unsuitable matrix of intensively managed agricultural areas (Brose 2001; De Meester *et al.* 2005). Kettle holes constitute hotspots of floral and faunal diversity in this landscape (Céréghino *et al.* 2012; Patzig *et al.* 2012) but are exposed to pollution and potential habitat destruction due to intensive agricultural practices in their immediate surrounding (Kalettka *et al.* 2001; Céréghino *et al.* 2008).

We selected four typical wetland plant species with different dispersion and reproductive traits: *Typha latifolia* L. (Typhaceae), *Phragmites australis* (Cav.) Trin. ex Steud. (Poaceae), *Lycopus europaeus* L. (Lamiaceae), and *Oenanthe aquatica* (L.) Poir (Apiaceae). The four species are hygrophilous with a frequent occurrence in kettle hole ecosystems and represent different degrees of clonality. The first two species are wind dispersed (pollen and seeds), while the latter two are insect-pollinated with seeds mainly dispersed by water (hydrochorous; Kühn *et al.* 2004). All species have mixed mating systems and reproduce both clonally and sexually with the exception of *Oenanthe aquatica*, which is mainly outcrossing (Favre-Bac *et al.* 2016) and reproduces mainly by seeds, although rhizomatous shoots have been observed (Hroudova *et al.* 1992).

We expected to find the lowest genetic divergence and highest within-population genetic diversity in the mainly outcrossing *Oenanthe aquatica* compared with the three plant species with mixed reproductive systems (asexual as well as sexual reproduction). We expected that predominantly selfing and clonal species possess a lower potential for dispersal and therefore show higher genetic divergence and less connectedness when compared with the mainly outcrossing species. This collection of species also allows the elucidation of how dispersal mechanisms influence genetic structure whereby wind dispersed species—*Typha latifolia* and *Phragmites australis*—would be expected to show higher rates of genetic exchange among populations compared with the hydrochorous species *Lycopus europaeus* and *Oenanthe aquatica*. Consequently, and with an opposing effect relative to our first hypothesis, the latter two species would be expected to show a higher genetic divergence and higher number of genetic clusters (higher differentiation among populations) and are expected to show an isolation-by-distance pattern as a result of restricted seed dispersal. In this sense, if the dispersal mechanisms are essential, we expect the landscape factors such as patch size and isolation to affect all species, but with a smaller effect in the wind dispersed species compared to the animal-pollinated/water-dispersed ones.

Oenanthe aquatica L. (Apiaceae) commonly named fine-leaved water Dropwort, is a colonizer species distributed in Eurasia typically occurring on the margins of shallow pools, ponds and ditches (Westberg *et al.* 2010). It is an annual, biennial or perennial species well-adapted to naturally nutrient-rich habitats with unpredictable fluctuations of water levels, sometimes overgrowing and becoming an undesirable weed under optimal conditions (Hroudova *et al.* 1992). It has a mixed breeding system where both outcrossing and selfing are common (Kühn *et al.* 2004); however, it was recently identified as mainly outcrossing (Favre-Bac *et al.* 2016). This species is diploid with 2n=22 chromosomes (Kühn *et al.* 2004). In the study area, *Oenanthe aquatica* is mainly restricted to kettle holes.

Lycopus europaeus L. (Lamiaceae) commonly called Gypsyworth is a perennial species distributed from Europe to Eastern Asia (China, Japan) and North Africa and naturally occurring along the banks of flowing-water ditches (Favre-Bac *et al.* 2016). It is a facultative xenogamous species (mainly outcrossing, but selfing is possible), with a competitor/stress tolerator ecological strategy (cs; Kühn *et al.* 2004). Like *O. aquatica, L. europaeus* is diploid with 2n=22 chromosomes (Kühn *et al.* 2004). In the study area, *Lycopus europaeus* is restricted to kettle holes.

Typha latifolia L. (broadleaf cattail, Typhaceae) is world-wide distributed, considered native in North and South America, Europe, Eurasia, and Africa, and reported as an invasive species in Hawaii and Australia (Champion *et al.* 2007; Gucker 2008). This species grows in fresh and brackish water, deep marshes, or shallow roadside ditches (Tsyusko *et al.* 2005). *Typha latifolia* is a perennial with a facultative autogamous breeding system (mainly selfing, but outcrossing is possible); with an "initial seedling recruitment" (ISR) ecological strategy (Kühn *et al.* 2004; Tsyusko *et al.* 2005). This species is diploid with 2n=30 chromosomes (Kühn *et al.* 2004). In the study area, *Typha latifolia* is not restricted to the kettle holes, occurring also in small and big lakes.

Phragmites australis (Cav.) Trin. ex Steud. (common reed, Poaceae) is a cosmopolitan grass species occurring mainly in wetlands, with native populations distributed in temperate zones on every continent except Antarctica. Some genotypes introduced from Europe have become invasive in North America (Packer *et al.* 2017). This species is a perennial helophyte with an extensive system of stout, underground rhizomes, with a mixed breeding system reproducing mostly vegetatively and rarely by seeds. The species is polyploid with diploid, triploid, tetraploid and octaploid individuals (n=12), with tetraploids being most common worldwide (Kühn *et al.* 2004; Saltonstall *et al.* 2007). In the study area, *Phragmites australis* is not restricted to kettle holes, occurring also in small and big lakes.

In *Oenanthe aquatica* and *Lycopus europaeus*, pollen transfer is mediated by insects and seed dispersal by water, while in *Typha latifolia* and *Phragmites australis* pollen and seeds are adapted to wind dispersal (Kühn *et al.* 2004; Kleyer *et al.* 2008). Occasionally, seeds can be dispersed by animals, mainly water birds through exozoochory in *Typha* and *Phragmites* and endozoochory in *Lycopus* and *Oenanthe* (Hroudova *et al.* 1992; Green *et al.* 2008; Brochet *et al.* 2009; Kleyheeg *et al.* 2015). *Lycopus europaeus* develops clonal rhizomes and stem tubers whereas *Typha latifolia* and *Phragmites australis* possess underground lateral rhizomes (Dickerman and Wetzel 1985; Packer *et al.* 2017). In *Oenanthe aquatica*, vegetative growth through rhizomes is possible but not common (Kühn *et al.* 2004; Klimešová and Bello 2009).

3.3 Methods

3.3.1 Study area

The study area was located in the Uckermark near Prenzlau in the so-called "AgroScapeLab Quillow". This agricultural landscape laboratory located at the Quillow river catchment area approx. 100 km North of Berlin (Germany, Brandenburg), is an open research platform established by the Leibniz Centre for Agricultural Landscape Research (ZALF). The water regime of the kettle holes in this region is influenced by a sub-humid climate with precipitation of 450–600 mm year^{-1} and potential evapotranspiration of 600–650 mm year^{-1} (Kalettka and Rudat 2006). This area comprises around 290 km^2 with intensive agriculture (65% of the land use) of maize, wheat and rapeseed and a high density of kettle holes (up to 2 per km^2). In total, 20 kettle holes were selected to sample populations of the four selected species (Figure 3.1, Table 3.2).

3.3.2 Sampling

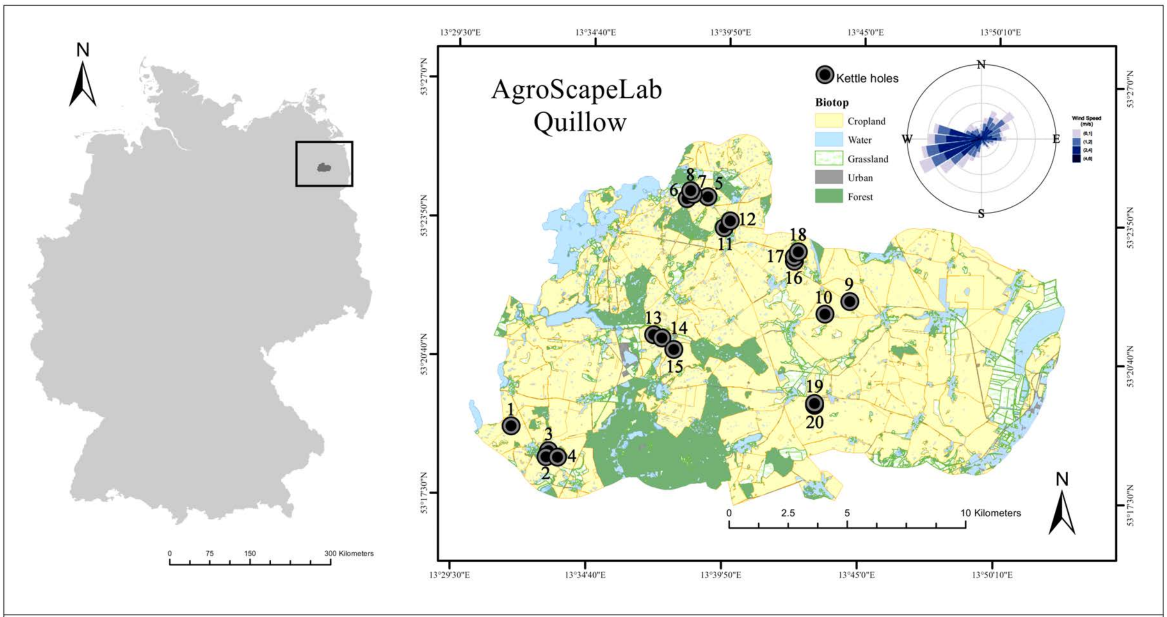
The selection of the kettle holes was based on an existing data set from ZALF to ensure a maximum connectivity by wind. Leaf samples from 10–25 individuals per species were collected in summer 2016 in a total of 20 kettle holes. The occurrence and number of individuals varied among the kettle holes (Table 3.2). Entire leaves were collected from *O. aquatica* and *L. europaeus* and between 5–10 cm tips were cut from *T. latifolia* and *P. australis*. Sample material was stored in tea bags and dried with silica gel prior to the laboratory work. Due to the clonal reproduction of the species, we maximised the distance between individuals to minimize the chance of collecting the same genet twice. *Oenanthe aquatica* and *L. europaeus* grow in clearly defined patches within the kettle hole while *T. latifolia* and *P. australis* usually grow in a single patch occupying a large fraction of the kettle hole. For these latter two species, we subdivided the large patch in five regions with a minimum pairwise distance of six meters among sites where leaves were sampled (e.g., Figure B8).

Additionally, we recorded presence and absence of all plant species occurring in the amphibian and terrestrial zone of the kettle hole (following Lozada-Gobilard *et al.* 2019). For an approximation of pollinator availability (Table 3.2), a list of bee species was taken from a parallel study (Lozada-Gobilard, unpublished data). Lists of bee and plant species can be found in Table B1.

3.3.3 DNA isolation and microsatellite genotyping

For DNA isolation, 12–20 mg of dried plant material was disrupted using a high-speed shaking instrument (TissueLyser QIAGEN) at 26.5 hertz for 4 minutes. Genomic DNA was extracted using the NucleoSpin 96 plant II kit (MACHEREY-NAGEL, Duren, Germany). The main steps of the standard protocol started with a washing step in 400 μl of Buffer PL1 and 10 μl of RNase A and incubation at 65° for 10 minutes. Subsequent clearing of impurities and filter was performed with 450 μl of Buffer PC and washing with 400 μl buffer PW1. For the final elution of DNA, 50 μl Buffer PE were added to the NucleoSpin Plant II Column and DNA concentration was measured with a NanoDrop instrument (NanoDrop 1000 spectrophotometer, Peqlab).

For microsatellite amplification, we used already published species-specific primers. We tested different primers until achieving a minimum of 10 polymorphic markers per species in a subset of 10–15 samples. All the primer pairs that worked in the test were selected and the forward primer of each pair was fluorescent labelled (M13-FAM). Locus-specific pairs of primers combined with 1 μl of DNA were used for the Polymerase Chain Reactions (PCR) using GoTaq polymerase. PCR reactions varied according to the specific references where the primers were taken from (Table B2). PCR products were sequenced using an "ABI Prism 3130xl Genetic Analyser" to confirm the presence of repetitive motives. Afterwards, PCR products were diluted 1:20 or 1:40 according concentrations of PCR product



Populations

1. Boi_28
2. Buc_1598
3. Buc_1604
4. Buc_2565
5. Dam_892
6. Dam_907
7. Dam_910
8. Dam_911
9. Fal_183
10. Fal_190
11. Kra_805
12. Kra_807
13. Raa_1189
14. Raa_2484
15. Raa_607
16. Rit_258
17. Rit_259
18. Rit_275
19. Wil_235
20. Wil_236

Fig. 3.1. Study area Agricultural Landscape Laboratory "AgroScapeLab Quillow" in the Quillow catchment area located in north-eastern Germany (Brandenburg). This agricultural landscape is characterized by a high density of kettle holes. Points denote our selected kettle holes. The wind rose on the upper right shows the speed and wind directions in the area. Main wind direction is from SW to NE with a maximum speed of 4.6 m/s. Percentage of land use in the area is 65% cropland, 17% forest, 9% grassland, 5% water, and 4% urban.

in the agarose gel, 0.25 μl dye-labeled size standard LIZ® was added and sequenced with 3130xl Genetic Analyser (Applied Biosystems® GeneticAnalyzers). Finally, allele size scoring was performed using GeneMapper® Software with the corresponding library of "bins" and double-checked by eye. The allelic data was exported to the software Excel (Microsoft Excel 2015 Version 15.13.4) (Link DOI Zalf [10.4228/ZALF.DK.110]). Due to the polyploid nature (mostly tetraploid) of *Phragmites australis*, we found one to four alleles per locus.

Prior to the statistical analyses, individuals with genotype information missing at three or more microsatellite loci were excluded. All analyses were conducted with repeated multilocus genotypes (i.e., clones) included and excluded to assess the effect on results of the genetic analyses.

3.3.4 Genetic diversity and genotypic richness

General genetic diversity measures of mean number of alleles, effective number of alleles, observed and expected heterozygosity were calculated using GenAlEx (Version 6.503; (Peakall and Smouse 2012). Since we lacked information on which alleles belonged to which genome for the allopolyploid, *Phragmites australis* was treated as autopolyploid and the data was analyzed accordingly (Meirmans *et al.* 2018). Following these authors, we assessed genetic diversity by calculating the expected heterozygosity analogous to diploid species by considering and weighting the different possible allelic combinations of partial heterozygotes to calculate observed heterozygosity. Specifically, observed heterozygosity was calculated based on the concept of "gametic heterozygosity" where the frequency of heterozygotes among randomly sampled diploid gametes is estimated based on the 4 allele copies at a locus, taking into account full and partial heterozygotes (Meirmans *et al.* 2018). Based on this, genetic parameters of expected heterozygosity (H_E) and observed heterozygosity (H_O), as well as inbreeding coefficient (F_{IS}) were calculated allowing us to compare the polyploid species with the other three diploid species.

To assess the extent of clonality, multilocus genotype matches among all individuals were counted with GenAlEx. Samples with identical genotypes but missing data for a locus were handled as a different genotype. We calculated the total number of unique genotypes (G) and genotypic richness R = (G-1)/(n-1) where n=is the number of individuals sampled per population (Dorken and Eckert 2001). A maximum genotypic richness of 1 means a complete absence of individuals of the same clone. Genetic diversity (mean and effective number of alleles, H_E and H_O) was very similar between datasets containing all samples (ramets) and only unique genotypes (genets), therefore we report results including clones (ramets).

3.3.5 F_{IS}, Multilocus Linkage disequilibrium (r_d) and Hardy-Weinberg equilibrium

Inbreeding coefficients (F_{IS}) were calculated using GenAlEx (Version 6.503; Peakall and Smouse 2012) and tested for a positive deviation from zero ($P < 0.05$) by comparing observed values to those obtained from 10,000 random permutations, as implemented in Arlequin (Version 3.5.2.2; Lischer and Excoffier 2010).

Tests of Hardy-Weinberg equilibrium (HWE) per locus were performed in Arlequin and

Multilocus Linkage Disequilibrium (r_drd) was assessed using the software Multilocus (Agapow and Burt 2001). Multilocus Linkage Disequilibrium (r_d) was separately calculated per species for each population based on two datasets: i) microsatellite data of the three diploid species and ii) a binary conversion of this dataset for comparison purposes with the tetraploid species. Binary data was taken from microsatellites data converting values of size of alleles into 0-1 presence/absence. The level of significance for r_d was adjusted by a Bonferroni correction. Since results between r_d calculated from microsatellite and binary data did not differ in *Oenanthe*, *Lycopus* and *Typha* (Figure B1), only r_d from binary data including *Phragmites* is reported.

Inbreeding coefficients (F_{IS}) were calculated separately for two datasets containing all samples (ramets) and only unique genotypes (genets) to assess a putuative overestimation of F_{IS} and r_d in the presence of clonal individuals. Evidence of Multilocus Linkage Disequilibrium (r_d) in combination with negative F_{IS} in both ramets and genets are an indication of sustained clonal growth in a population (Halkett *et al.* 2005).

Additionally, we applied ANOVAs in combination with post-hoc Tukey Tests to evaluate significant differences in mean number of alleles, observed and expected heterozygosity, gametic richness, F_{IS} and r_d among species using the functions “anova” and “TukeyHSD” from the package stats in R (2018).

3.3.6 AMOVA

To partition the genetic variation within and among kettle holes, an Analysis of Molecular Variance (AMOVA) was performed using Arlequin (Version 3.5.2.2; Lischer and Excoffier 2010). Since there is no possibility of performing an AMOVA with tetraploid allelic data of *P. australis*, we converted our microsatellite data to “allelic phenotypes" based on the presence and absence of alleles. This 0–1 data matrix was used for AMOVA analysis following Fér and Hroudová (2009).

3.3.7 Genetic differentiation and structure

We used a Bayesian clustering algorithm implemented in STRUCTURE to explore population structure (Pritchard *et al.* 2000) following an "Admixture Model" with correlated allele frequencies. The range of possible clusters (K) was set from 1–11. The length of the burn-in period was set to 100,000, followed by 2,000,000 iterations with 30 replicates at each K (Porras-Hurtado *et al.* 2013). Structure output was analyzed using STRUCTURE HARVESTER (Earl and VonHoldt 2012), which estimates the optimal number of clusters by identifying the highest DeltaK value (Evanno *et al.* 2005). To estimate the optimal clustering, as well as the individual membership within clusters, CLUMPAK (Cluster Markov Packager Across K) was used (Kopelman *et al.* 2015).

Since *P. australis* is an allotetraploid with a disomic inheritance pattern (Soltis and Soltis 2000; Saltonstall 2003), we recoded allele codes as follows: (1) one allele at a locus counted as four identical alleles; (2) two alleles at a locus counted as each allele occurring twice; (3) three alleles at a locus counted as each allele occurring once and a fourth allele as missing data; (4) four alleles at a locus counted as each allele occurring once following Fér and

Hroudová (2009). This enabled us to analyze our data with STRUCTURE, which can handle tetraploid data (Pritchard *et al.* 2000).

Data conversion between the various software used was carried out with PGDSpider (Version 2.1.1.5; Lischer and Excoffier 2012).

3.3.8 Analysis of dispersal vectors and genetic divergence

Pairwise F_{ST} and Jost D (for loci with high allelic diversity) were calculated as a measure of genetic distance between populations. To evaluate if the geographic distance between kettle holes was correlated with genetic distance between populations (isolation-by-distance; IBD), we applied Mantel tests with 10,000 permutations. Additionally, we conducted Spatial Autocorrelation Analyses. Two analyses were run for each species with either 3 or 5 even distance classes using GenAlEx (Version 6.503; Peakall and Smouse 2012).

In addition, we evaluated the effect of wind and the presence of pollinators (bees) on population differentiation in the two wind-dispersed species (*P. australis* and *T. latifolia*) and the insect-pollinated species (*L. europaeus* and *O. aquatica*). First, we compared estimates of F_{ST} and Jost D to a weighted matrix based on direction and speed of wind corresponding to the area of study (Table B7). Similarly, a presence/absence matrix of bee species known as pollinators of our plants was compared to the genetic distance matrices (Table B8). To account for the location of the kettle holes, Partial Mantel test were performed based on 10,000 permutations.

F_{ST} and Jost D matrices for *P. australis* were calculated with the package "polysat" and Mantel and Partial Mantel tests were performed with the package "vegan" in R (Version 3.4.3; R. 2018).

3.3.9 Analysis of genetic diversity and landscape factors

We used Linear Models to test for the effects of several landscape-related factors on genetic diversity (expected and observed heterozygosity) and inbreeding coefficient (F_{IS}). An initial model was fitted to evaluate the effect of dispersal mode and species identity on genetic diversity and inbreeding coefficient. Based on those results, we built a general model including dispersal mode (wind vs. insect), species identity, biotic and abiotic factors (see below), and all interactions (clonal vs. non-clonal was not tested, since only one outbreeding species vs. clonal three). Abiotic factors included area (patch size), number of neighboring kettle holes within a buffer of 500 m (degree of isolation) based on a previous study in the area that determined 500 m as the most important buffer zone (Lozada-Gobilard *et al.* 2019). Biotic factors included species richness of bees as pollinator resource-availability and species richness of plants occurring in the kettle holes as an approximation of habitat heterogeneity. After a forward selection, a final most parsimonious model was selected based on Akaike's information criterion (AIC). Additionally, each species was analyzed separately to disentangle the effect of each factor on genetic diversity and F_{IS}.

All statistical tests were performed using the packages MASS and effects in R version 3.14 (R. 2018).

3.4 Results

In total, 20 kettle holes were sampled, but not all study species were present in each kettle hole (Table 3.2). In these 20 kettle holes, 752 individuals were genotyped from a total 20 populations with a minimum of eight and maximum of 12 populations per species (Table 3.2). Clonal individuals were found in all four species with the lowest number in *Oenanthe aquatica*. From the total number of individuals per species, we identified 20 (43%), 23 (48%), and 24 (20%) clonal individuals in *Typha latifolia*, *Phragmites australis* and *Lycopus europaeus* (respectively); but only 7 (2%) clonal individuals in *Oenanthe aquatica* (Table 3.2).

3.4.1 Genetic diversity

Genetic diversity in terms of effective number of alleles (ENA), gametic richness (R), observed heterozygosity (H_O) and expected heterozygosity (H_E) varied significantly among species (ANOVA tests $F_{3,32}$=20.36 (ENA), $F_{3,32}$=26.30 (R), $F_{3,32}$=19.43 (H_O), $F_{3,32}$=37.08 (H_E); all $P<0.001$) with the highest values found in *Oenanthe aquatica* and lowest in *Typha latifolia* (Figure 3.2, Table 3.3). Highest percentage of unique genotypes was found in *O. aquatica* (mean: 98%) and lowest (52%) in *P. australis* and *T. latifolia* (57%). *Phragmites australis* and *T. latifolia* showed low gametic richness (both species: 0.5) differing significantly from both *L. europaeus* (0.8) and *O. aquatica* (0.9) (Figure 3.2, Table 3.3).

Genetic diversity H_O and H_E was best explained by species identity (65 and 78% of explained variability respectively), with a significant effect of landscape abiotic factors (patch size and isolation) and bee richness only on H_O (Table 3.4). Interactions of species with these factors (Table 3.4 [a:c] = Species: Plant richness; [a:d] = Species: Bee richness, [a:e] = Species: Isolation) were also significant for H_O, while H_E was only influenced by the interaction species identity and plant richness (Table 3.4).

Landscape factors affected genetic diversity (H_O and H_E) differently among species (Figure B6, B7 respectively). In *Oenanthe*, H_O was significantly or marginally affected by patch size, degree of isolation and plant richness whereas H_E was only significantly affected by plant-species richness. In *Lycopus*, H_O was only affected by the degree of isolation, while genetic diversity indices of *Typha* and *Phragmites* were in general not affected by any factor (Table 3.4, Figure B6, B7).

TABLE 3.2: **Landscape characteristics and number of individuals per species sampled in the kettle holes.** Kettle-hole location and environmental factors important for connectivity are shown. Listed are the abiotic factors area in m^2, patch size, and the degree of isolation measured as the number of ponds occurring in a 500-m buffer as well as the biotic factors plant and bee-species richness (number of species) per kettle hole. In addition, information on the hydro-geomorphological (HGM) type of the kettle hole (S: Storage vs. SO: Storage Overflow) and surrounding land use (R: Rapeseed, C: Cereal and G: Grassland) is provided for each kettle hole. Occurrence of our studied species *Phragmites australis (Pa)*, *Typha latifolia (Tl)*, *Oenanthe aquatica (Oa)* and *Lycopus europaeus (Le)* varied among kettle holes.

n	**Pop**	**ID Pond**	**Region**	**Longitude**	**Latitude**	**Area [m^2]**	**Buffer**	**Plant sp**	**Bee sp**	**HGM**	**Land use**	**No of samples**			
												Pa	***Tl***	***Oa***	***Le***
1	Boi_28	28	Boisterfelde	13.528872	53.31775	4896	12	70	8	S	R	–	23	25	9
2	Buc_1598	1598	Buchenhain	13.553018	53.30854	3007	19	42	20	S	C	–	25	19	12
3	Buc_1604	1604	Buchenhain	13.551442	53.30617	1534	13	45	4	SO	C	15	22	–	26
4	Buc_2565	2565	Buchenhain	13.558765	53.30618	7097	20	61	11	S	R	–	–	13	33
5	Dam_892	892	Damerow	13.651345	53.40636	3831	18	48	25	SO	R	25	–	–	–
6	Dam_907	907	Damerow	13.638137	53.40552	574	17	37	12	SO	G	–	9	–	5
7	Dam_910	910	Damerow	13.641936	53.40719	1020	13	33	14	SO	G	–	–	25	–
8	Dam_911	911	Damerow	13.640171	53.40855	2328	13	40	13	SO	G	–	24	–	22
9	Fal_183	183	Falkenhagen	13.742717	53.36747	327	12	41	19	S	C	25	–	–	–
10	Fal_190	190	Falkenhagen	13.727117	53.36244	2651	5	33	10	S	C	23	–	–	20
11	Kra_805	805	Kraatz	13.662017	53.39467	3198	21	50	11	SO	R	24	10	25	12
12	Kra_807	807	Kraatz	13.665798	53.39738	1470	17	46	11	S	R	–	–	25	–
13	Raa_1189	1189	Raakow	13.618345	53.35352	1049	24	23	14	S	C	–	–	25	–
14	Raa_2484	2484	Raakow	13.623681	53.35228	14850	22	30	7	SO	C	25	25	–	–
15	Raa_607	607	Raakow	13.631487	53.34798	3914	29	31	5	SO	C	25	–	–	–
16	Rit_258	258	Rittgarten	13.707032	53.38247	4284	14	29	10	SO	C	–	–	27	–
17	Rit_259	259	Rittgarten	13.706979	53.38409	1045	17	29	11	SO	C	25	–	25	–
18	Rit_275	275	Rittgarten	13.709487	53.38598	4398	22	45	16	SO	C	–	16	25	–

Table 3.2 continued from previous page

19	Wil_235	235	Wilhelmshof	13.721543	53.32781	1446	6	34	9	S	C	–	–	26	–
20	Wil_236	236	Wilhelmshof	13.721488	53.32848	440	6	25	9	SO	C	–	–	12	–
	TOTAL	**(ramets)**										**187**	**154**	**272**	**139**
		(genets)										**164**	**134**	**265**	**115**

TABLE 3.3: **Sample size (N) and summary of basic genetic diversity parameters for 13, 15, 10, and 10 nuclear microsatellites among populations of *Oenanthe aquatica* (Oa), *Lycopus europaeus* (Le), *Typha latifolia* (Tl), and *Phragmites australis* (Pa), respectively.** Genetic diversity parameters analyzed include mean number of alleles (MNA), effective number of alleles (ENA), Shannon-Index of diversity (I), observed (H_O) and expected heterozygosity (H_E). To assess clonal diversity, the number of unique genotypes (G) and the genotypic richness R = (G-1)/(n-1) were calculated. An inbreeding coefficient (F_{IS}) was calculated to measure departures from panmixia within populations. "F_{IS} ramets" corresponds to all individuals including clones, while "F_{IS} genets" excludes clonal individuals. Significance values correspond to populations deviating from Hardy-Weinberg equilibrium. Due to polyploidy of *P. australis*, tests of significance were not calculated. For comparison purposes with *P. australis*, multilocus linkage disequilibrium r d was calculated based on binary data. Significance levels are indicated with asterisks: ***$P < 0.001$, ** $P < 0.01$, * $P < 0.05$, "."$P = 0.05$.

	Pop	**N**	**MNA**	**ENA**	**H_O**	**H_E**	**G**	**R**	**F_{IS} (ramets)**	**F_{IS} (genets)**	**r_d**
Oa	Boi_28	25	3.69 ± 0.40	1.85 ± 0.16	0.34 ± 0.05	0.41 ± 0.05	24 (96%)	0.96	0.21***	0.19***	0.03***
	Buc_1598	19	5 ± 0.58	3.25 ± 0.39	0.56 ± 0.05	0.63 ± 0.04	19 (100%)	1	0.14**	0.14***	0.01**
	Buc_2565	13	4.54 ± 0.61	3.15 ± 0.46	0.56 ± 0.07	0.6 ± 0.06	13 (100%)	1	0.12*	0.12**	0
	Dam_910	25	5.69 ± 0.73	2.97 ± 0.40	0.6 ± 0.06	0.6 ± 0.04	25 (100%)	1	0.01	0.01	0.00*
	Kra_805	25	6.62 ± 0.84	3.51 ± 0.58	0.63 ± 0.04	0.66 ± 0.03	25 (100%)	1	0.07*	0.07*	0.01**
	Kra_807	25	5.08 ± 0.67	3.09 ± 0.35	0.6 ± 0.05	0.63 ± 0.03	24 (96%)	0.96	0.07.	0.08.	0
	Raa_1189	24.9	5.85 ± 0.86	3.32 ± 0.42	0.64 ± 0.05	0.64 ± 0.05	25 (100%)	1	0.01	0.01	0
	Rit_258	26.9	6.69 ± 0.77	3.79 ± 0.43	0.68 ± 0.04	0.69 ± 0.04	27 (100%)	1	0.04	0.04	0
	Rit_259	25	5.92 ± 0.49	3.68 ± 0.38	0.66 ± 0.04	0.69 ± 0.03	22 (88%)	0.88	0.06	0.08*	0.00*
	Rit_275	25	5.46 ± 0.63	2.63 ± 0.33	0.53 ± 0.05	0.55 ± 0.05	25 (100%)	1	0.07*	0.07*	0
	Wil_235	26	5.62 ± 0.71	3.18 ± 0.39	0.59 ± 0.04	0.63 ± 0.04	24 (92%)	0.92	0.08*	0.07*	0

Table 3.3 continued from previous page

	Wil_236	12	4.23 ± 0.47	2.51 ± 0.23	0.58 ± 0.04	0.55 ± 0.04	12 (100%)	1.00	0.00	0.00	0.00
Le	Boi_28	9	3.33 ± 0.33	2.57 ± 0.27	0.58 ± 0.06	0.53 ± 0.06	8 (89%)	0.88	-0.03	-0.03	0.07***
	Buc_1598	12	2.73 ± 0.25	2.27 ± 0.19	0.38 ± 0.08	0.51 ± 0.05	9 (75%)	0.73	0.29**	0.22	0.20***
	Buc_1604	26	2.73 ± 0.21	1.96 ± 0.18	0.42 ± 0.07	0.42 ± 0.05	16 (62%)	0.6	0.02	0.03	0.08***
	Buc_2565	32.9	3.87 ± 0.27	2.11 ± 0.17	0.28 ± 0.05	0.48 ± 0.04	21 (64%)	0.63	0.43***	0.43***	0.07***
	Dam_907	5	2.2 ± 0.24	1.76 ± 0.18	0.41 ± 0.12	0.34 ± 0.07	5 (100%)	1	-0.12	-0.12	0.47***
	Dam_911	22	3.27 ± 0.38	2.14 ± 0.17	0.49 ± 0.08	0.48 ± 0.05	17 (77%)	0.76	-0.01	0.03	0.07***
	Fal_190	20	2.67 ± 0.27	1.77 ± 0.12	0.44 ± 0.08	0.39 ± 0.04	16 (80%)	0.79	-0.08	-0.03	0.08***
	Kra_805	12	2.4 ± 0.31	1.79 ± 0.17	0.16 ± 0.08	0.36 ± 0.06	11 (92%)	0.91	0.60***	0.61***	0.19
Tl	Boi_28	22.8	2.5 ± 0.62	1.63 ± 0.36	0.17 ± 0.10	0.21 ± 0.09	14 (61%)	0.6	0.24**	0.25***	0.14***
	Buc_1598	24.8	2 ± 0.21	1.7 ± 0.21	0.49 ± 0.14	0.32 ± 0.08	14 (56%)	0.55	-0.51***	0.25***	0.16***
	Buc_1604	22	2.8 ± 0.51	1.65 ± 0.35	0.19 ± 0.08	0.25 ± 0.09	16 (73%)	0.71	0.21**	-0.49***	0.09**
	Dam_907	9	1.7 ± 0.26	1.42 ± 0.19	0.28 ± 0.13	0.2 ± 0.08	4 (44%)	0.38	-0.35.	-0.29	0.33***
	Dam_911	23.8	2.1 ± 0.41	1.55 ± 0.28	0.27 ± 0.11	0.23 ± 0.08	12 (50%)	0.48	-0.15***	-0.15*	0.21***
	Kra_805	10	1.4 ± 0.16	1.27 ± 0.13	0.22 ± 0.13	0.15 ± 0.07	4 (40%)	0.33	-0.42***	-0.39***	0.45*
	Raa_2484	24.6	2.2 ± 0.49	1.41 ± 0.19	0.11 ± 0.07	0.2 ± 0.08	14 (57%)	0.55	0.49***	0.49***	0.09***
	Rit_275	15.2	1.6 ± 0.31	1.3 ± 0.13	0.17 ± 0.10	0.17 ± 0.07	11 (72%)	0.7	-0.32***	-0.30***	0.06
Pa	Buc_1604	14.5	4.4 ± 1.65	3.35 ± 1.18	0.46 ± 0.26	0.66 ± 0.15	13 (90%)	0.89	0.3	0.31	0.17***
	Dam_892	25	2.3 ± 0.95	2.11 ± 0.87	0.51 ± 0.31	0.45 ± 0.25	7 (28%)	0.25	-0.15	-0.14	0.24**
	Fal_183	25	2 ± 0.94	1.99 ± 0.93	0.44 ± 0.34	0.39 ± 0.28	7 (28%)	0.25	-0.14	-0.13	0.20***
	Fal_190	22.5	3.1 ± 1.10	2.31 ± 0.80	0.47 ± 0.24	0.5 ± 0.23	14 (62%)	0.6	0.06	0.04	0.21***
	Kra_805	23.9	4.4 ± 2.22	3.09 ± 1.46	0.45 ± 0.27	0.59 ± 0.24	15 (63%)	0.61	0.23	0.23	0.14***
	Raa_607	24.6	4.7 ± 2.36	3.34 ± 1.89	0.44 ± 0.28	0.58 ± 0.29	14 (57%)	0.55	0	0.24	0.17***
	Raa_2484	24.7	3.1 ± 1.79	2.05 ± 1.03	0.38 ± 0.35	0.38 ± 0.32	10 (40%)	0.38	0.24	0	0.37***
	Rit_259	24.4	2.1 ± 1.20	1.78 ± 0.76	0.35 ± 0.31	0.33 ± 0.29	12 (49%)	0.47	-0.07	-0.06	0.30***

3.4.2 Inbreeding coefficient F_{IS}

Inbreeding coefficients were calculated for two datasets: one including all individuals (ramets) and one including only one genotype per clone (genets). Only small differences were observed between these datasets (Table 3.3). Even though, no significant differences in F_{IS} were found among species (Figure 3.2E), a significantly positive relationship of F_{IS} with

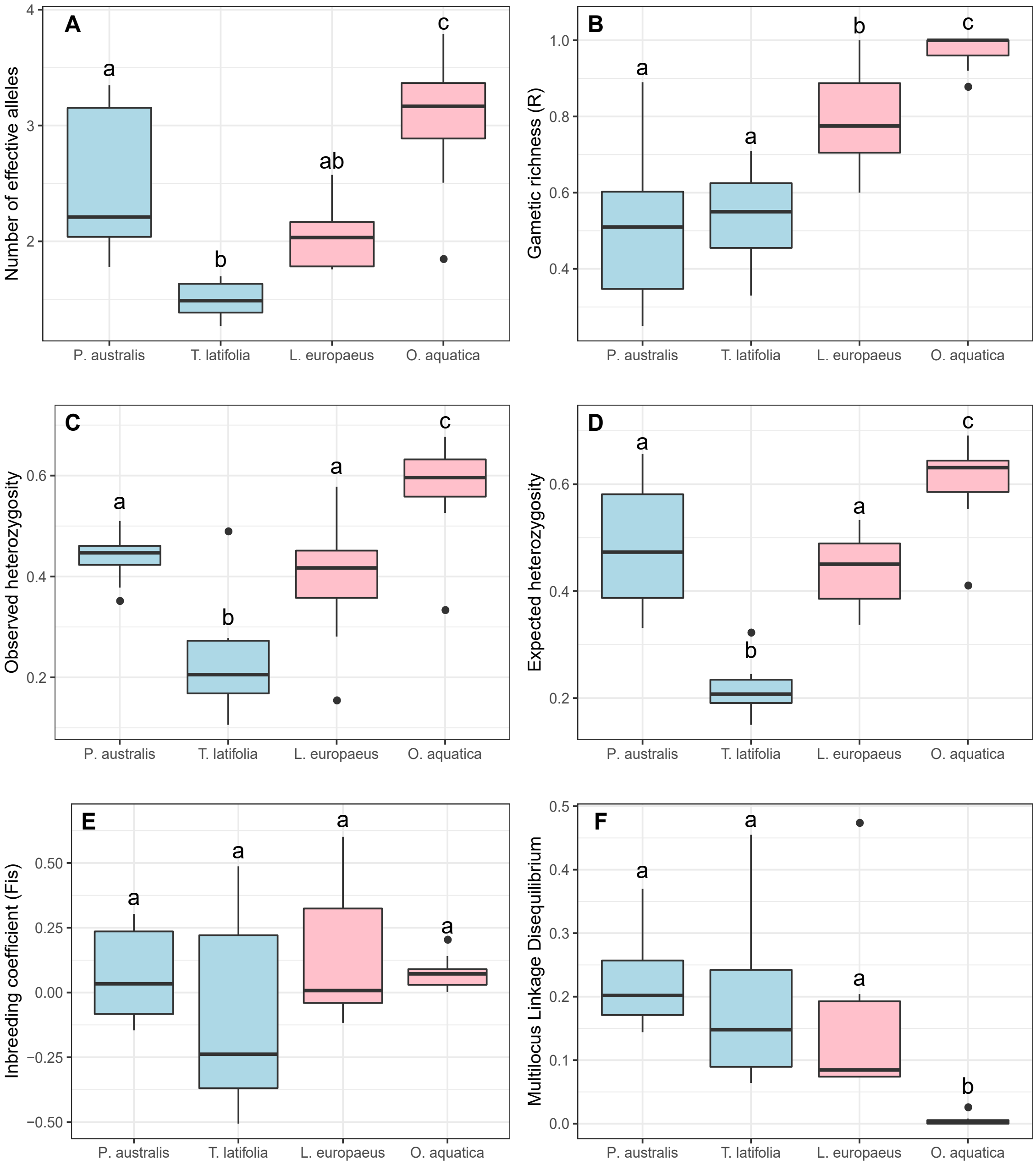


Fig. 3.2. Basic genetic diversity among populations of *Oenanthe aquatica* (insect pollinated), *Lycopus europaeus* (insect pollinated), *Typha latifolia* (wind pollinated), and *Phragmites australis* (wind pollinated). Main genetic diversity parameters include effective number of alleles (A), gametic richness calculated as R = (G-1)/(n-1) (B), observed heterozygosity (C), expected heterozygosity (D) F_{IS} Inbreeding coefficient (E) and Multilocus Linkage Disequilibrium (rd) (F). Different letters represent significant differences calculated with a Tukey Post-Hoc test. Blue: wind pollinated and dispersed, red: insect pollinated, water dispersed.

patch size (area) and plant-species richness per kettle hole was found for *O. aquatica* (Figure 3.3, Table 3.4).

In *Oenanthe*, 7 out of 12 populations showed significantly positive (albeit low) F_{IS} values, indicating some within-population inbreeding. In *Lycopus* only 2 out of 8 populations showed significantly positive F_{IS}. The mainly clonally reproducing *Typha* was characterized by significant and mostly negative F_{IS} in almost all populations (7/8). Significance tests on F_{IS} could not be performed for the tetraploid *Phragmites* (Table 3.3).

Species identity explained 13% of the total variation in F_{IS}, while the interaction Species: Isolation explained 18% (Table 6). Landscape factors such as patch size and bee-species richness were the main factors influencing F_{IS}. Patch size had a positive effect on F_{IS} in all species, albeit not significant in *Phragmites* (Table 3.4, Figure 3.3A). F_{IS} significantly increased when populations were less isolated (higher number of ponds in a 500m buffer) in *Lycopus* (Table 3.4, Figure 3.3B). In *Typha* and *Phragmites*, F_{IS} significantly decreased with increasing bee richness (Figure 3.3D), while F_{IS} increased with plant richness in *Oenanthe* (Figure 3.3C).

3.4.3 Multilocus Linkage Disequilibrium

Multilocus Linkage Disequilibrium (r_d) differed significantly among species ($F_{3,32}$=10.06, $P<0.001$). While in *O. aquatica* r_d was close to zero in all populations, indicating only small Linkage Disequilibrium (Table 3.3, Figure 3.2), significant and high r_d was detected in almost all populations of the remaining species. Negative population F_{IS} observed in *Lycopus*, *Typha* and *Phragmites* combined with significant Multilocus Linkage Disequilibrium indicated sustained clonal growth, which has been described to cause such a diversity pattern due to the accumulation of somatic mutations and reduced sexual recombination (Halkett *et al.* 2005).

3.4.4 Population differentiation and gene flow

Pairwise F_{ST} between populations ranged from 0.03–0.26 in *Oenanthe*, 0.22–0.47 in *Lycopus*, 0.19–0.67 in *Typha* and 0.03–0.15 in *Phragmites* (all significant at $P<0.05$ after Bonferroni correction; Table B3–B6). Higher global F_{ST} were found in *Lycopus* and *Typha* (0.37 and 0.39, respectively), compared to *Oenanthe* (0.14) based on microsatellites data (Table 3.6).

Distance matrices of pairwise F_{ST} and Jost D showed evidence of isolation by distance (IBD) for *Oenanthe* and *Lycopus*. No relationship was found with any other dispersal-related factor (neither related to wind direction or speed nor to species richness of bees; Table 3.5). Spatial autocorrelation analyzes showed significant spatial structure according to IBD ($P<0.001$), with a more pronounced spatial autocorrelation in *Lycopus*, *Typha*, and *Phragmites* compared to *Oenanthe*. There was a particularly steep decrease between the 3 and 6 km distance classes in *Phragmites* (Figure 3.4). In addition, spatial genetic structure in the insect-pollinated species, *Lycopus* and *Oenanthe* was found at larger geographic distances than the wind dispersed *Typha* and *Phragmites* (9 km vs. 6 km respectively; Figure 3.4).

TABLE 3.4: Relationship between genetic diversity (H_O and H_E) and inbreeding coefficient (F_{IS}) with abiotic (area and isolation) and biotic (plant and bee species richness) parameters important for landscape connectivity based on linear models. Variation in percentage was calculated based on the Sum of Squares after an ANOVA test. The Full Model shows which landscape factors contribute to the overall variation and the corresponding interactions, while "By species" displays the relationship separately by species. Significance levels are indicated with asterisks: ***P< 0.001, ** P< 0.01, * P< 0.05, "."P =0.05.

Response variable:		**H_O**			**H_E**			**F_{IS}**	
Predictors	**%**	**F**	**P-value**	**%**	**F**	**P-value**	**%**	**F**	**P-value**
FULL MODEL									
Species [a]	64.6	101.5	<0.001***	77.7	69.7	<0.001***	13	7.7	0.006**
Patch size (Log area) [b]	2.4	11.2	0.007**	0.2	0.4	0.54	17.1	30.2	<0.001***
Plant richness [c]	0.8	3.8	0.079	0.1	0.2	0.68	0.1	0.2	0.63
Bee richness [d]	1.9	9	0.013*	0.1	0.2	0.66	10.7	19	0.001**
Isolation (Buffer) [e]	1.4	6.5	0.029*	0.1	0.2	0.65	0.4	0.7	0.43
Region	2.6	2	0.16	5.8	2.6	0.088	4.7	1.4	0.31
[a:b]	1.4	2.3	0.14	1.6	1.4	0.3	9.3	5.5	0.018*
[a:c]	5.6	8.9	0.004**	4.7	4.2	0.036*	3.9	2.3	0.14
[a:d]	4.9	7.7	0.006**	3.2	2.9	0.089	16.8	9.9	0.002**
[a:e]	12.3	19.3	<0.001***	3	2.7	0.1	18.2	10.7	0.002**
Residuals	2.1			3.7			5.7		
Adjusted R-square	0.9258	(p<0.001)		0.424	(p<0.001)		0.801	(p=0.001)	
MODEL BY SPECIES									
Oenanthe aquatica									
Patch size (Log area)	15.1	5.2	0.057 (*)	3.1	0.6	0.47	44.6	15.9	0.005**
Plant richness	48.4	16.6	0.005**	44.6	8.5	0.022*	28	10	0.016*
Bee richness	0.1	0.1	0.83	0.1	0	0.9	1	0.4	0.57
Isolation (Buffer)	16	5.5	0.052 (*)	15.6	3	0.13	6.8	2.4	0.17
Residuals	20.4			36.6			19.7		

Table 3.4 continued from previous page

Response variable:	**H_O**			**H_E**			**F_{IS}**		
Adjusted R-square	0.678	(p=0.01)		0.424	(p=0.09)		0.69	(p=0.001)	
Lycopus europaeus									
Patch size (Log area)	2.2	0.4	0.57	45	4.4	0.13	29	12.1	0.04*
Plant richness	3.5	0.7	0.47	2.5	0.2	0.65	0.2	0.1	0.82
Bee richness	0.7	0.1	0.74	7.8	0.8	0.45	6.4	2.7	0.2
Isolation (Buffer)	78	15	0.031*	14.3	1.4	0.32	57.3	23.9	0.016*
Residuals	15.6			30.4			7.2		
Adjusted R-square	0.635	(p=0.14)		0.29	(p=0.34)		0.831	(p=0.04)	
Typha latifolia									
Patch size (Log area)	16.7	2.1	0.24	1.3	0.1	0.8	25.3	10.8	0.046*
Plant richness	1.1	0.1	0.74	1	0.1	0.82	0.1	0	0.85
Bee richness	52.4	6.6	0.083	11	0.7	0.46	58.4	25	0.015*
Isolation (Buffer)	5.9	0.7	0.45	40.2	2.6	0.21	9.2	3.9	0.14
Residuals	23.9			46.6			7		
Adjusted R-square	0.442	(p=0.24)		0.08	(p=0.57)		0.836	(p=0.04)	
Phragmites australis									
Patch size (Log area)	0.2	0	0.88	1.3	0.2	0.66	21.6	4.4	0.13
Plant richness	49.8	6.9	0.079	24.2	4.2	0.13	3.7	0.8	0.45
Bee richness	3	0.4	0.57	51	8.9	0.059 (*)	60.1	12.4	0.039*
Isolation (Buffer)	25.3	3.5	0.16	6.3	1.1	0.37	0.1	0	0.92
Residuals	21.7			17.2			14.6		
Adjusted R-square	0.493	(p=0.21)		0.598	(p=0.159)		0.659	(p=0.12)	

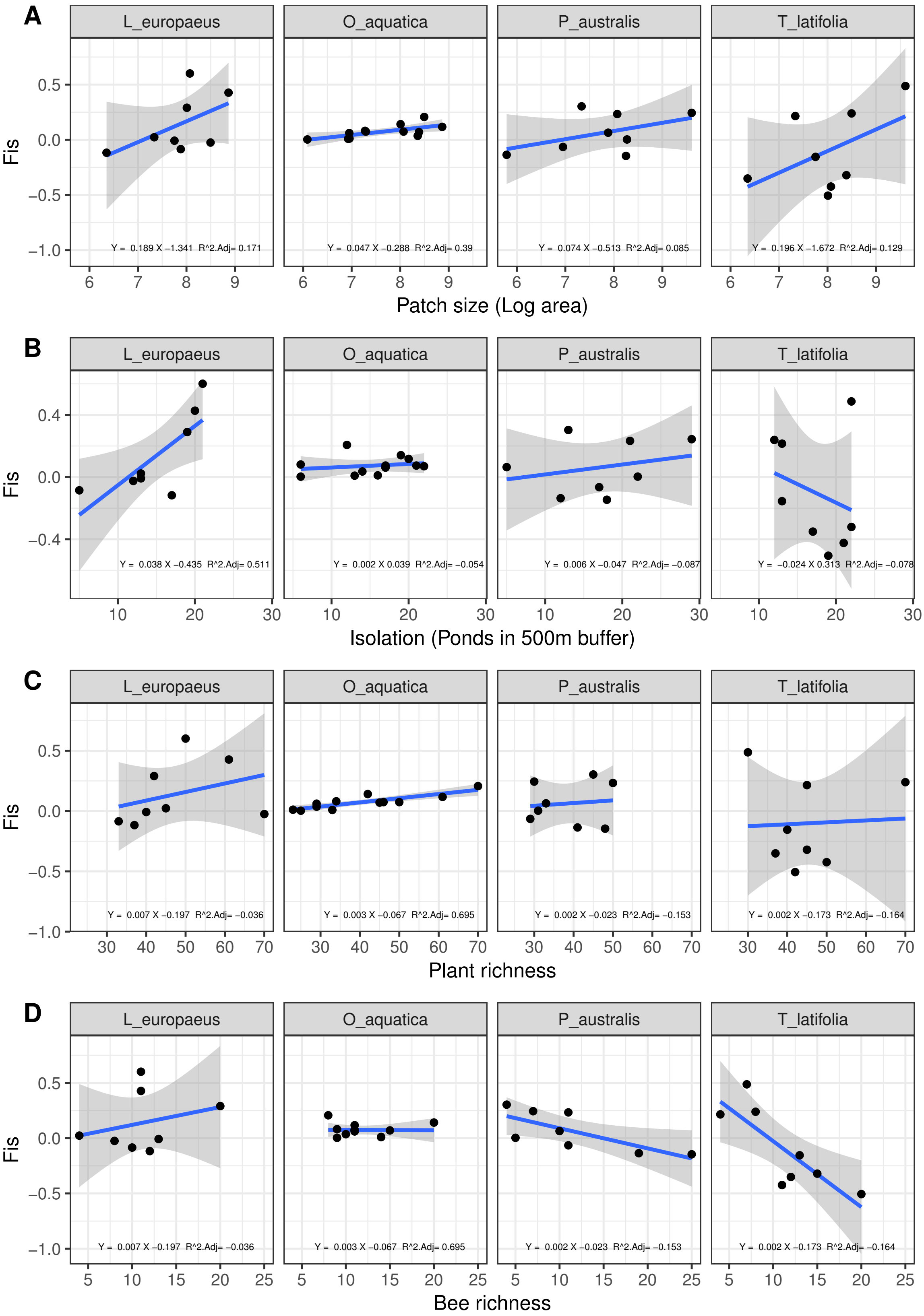


Fig. 3.3. Linear models representing the relationship between Inbreeding coefficient (F_{IS}) with the biotic and abiotic factors. Patch size represented as the Log area of the kettle hole (A), isolation degree depicted as the number of ponds occurring in a 500 m buffer (B), plant species richness measured in presence absence of plant species occurring in the kettle hole (C) and bee richness also measured in presence absence of bees species found in the kettle hole (D).

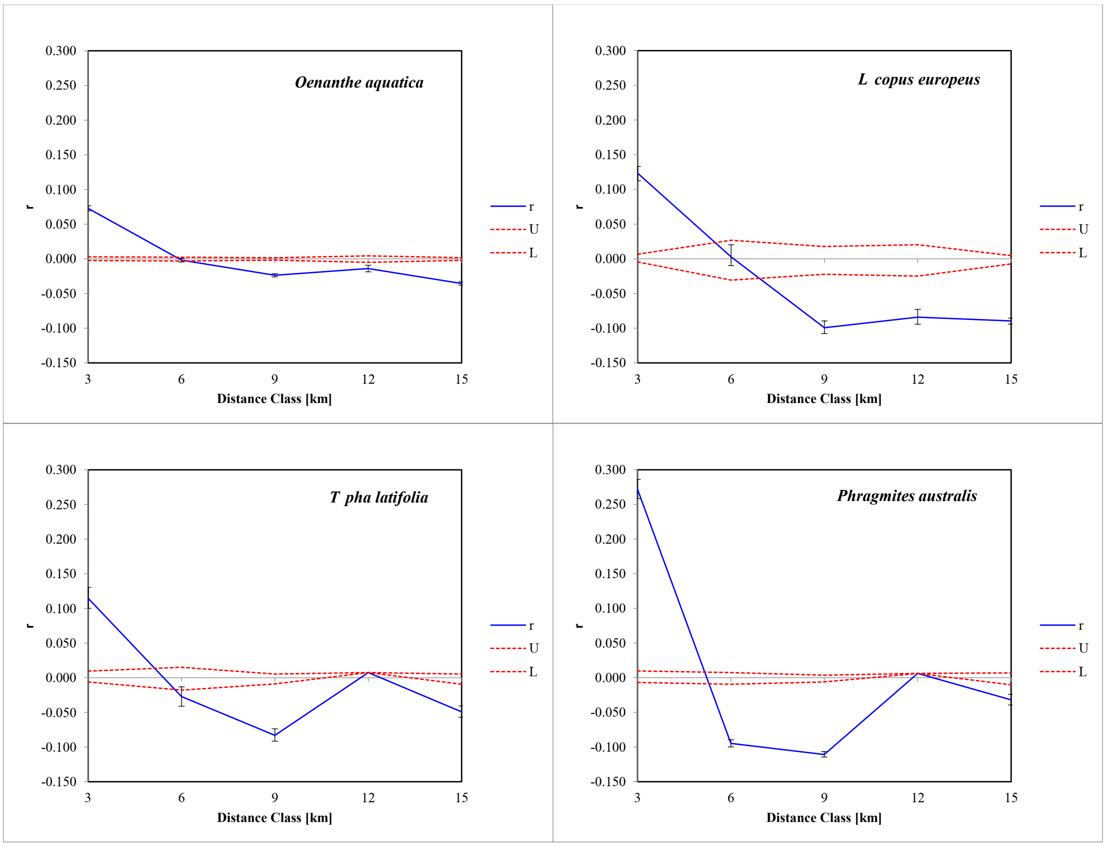


Fig. 3.4. Spatial Autocorrelation analyzes based on genetic and geographic distances taking a distance class separation of 3 km. Values corresponding to zero at distance class of 12 in *Typha latifolia* and *Phragmites australis* means that no populations were sampled at those distances. Upper (U) and lower (L) confidence limits bound the 95% confidence interval to test the null hypothesis of No spatial structure for the combined data set as determined by 999 permutations. All correlograms are significant (P<0.001) showing spatial structure for all species.

The Bayesian Structure analyzes suggested a higher number of genetic clusters in the insect-pollinated species *Oenanthe* and *Lycopus* (K=8 and K=7, respectively) than in the wind dispersed *Typha* and *Phragmites* (K=2 for both). Similar results were achieved when populations were analyzed excluding clones (*Oenanthe* K=6, *Lycopus* K=7, *Typha* K=3 and *Phragmites* K=2). Although there seemed to be a smaller number of genetic groups in the wind dispersed species *Typha* and *Phragmites* compared with *Lycopus* and *Oenanthe*, delta K values for the most probable number of genetic groups (K) was <30 in all species, indicating only weak genetic structuring within species (Figure B2–B5).

AMOVA results based on microsatellites data showed the highest percentage of variation within individuals in *Oenanthe* (80%) and lower variation among populations (*Oenanthe*: 14%) compared to *Lycopus* (37%) and *Typha* (40%). For binary data, the highest variation within populations was also found in *Oenanthe* (80%), as well as lower variation among populations (*Oenanthe*: 20%) compared to *Lycopus* (60%), *Typha* (60%) and *Phragmites* (64%) (Table 3.6).

TABLE 3.5: **Summary of the Mantel and Partial Mantel tests to evaluate the relationship between genetic distance and dispersal vectors.** Isolation-by-Distance Analyses were performed comparing Euclidean distances with genetic distances. Additionally, we compared a weighted matrix based on wind direction and speed to test for the wind effects and finally, we estimated the effect of pollinators represented by bee-species richness per kettle hole on genetic distance. These comparisons were based under the assumption that wind has a larger effect on the wind dispersed species:*Typha latifolia* and *Phragmites australis* and bee diversity influenced only the insect-pollinated species *Oenanthe aquatica* and *Lycopus europaeus*. Significance levels are indicated with asterisks (***P< 0.001, , ** P< 0.01).

	O. aquatica		***L. europaeus***		***T. latifolia***		***P. australis***	
	r	**P-value**	**r**	**P-value**	**r**	**P-value**	**r**	**P-value**
Mantel Fst								
IBD	0.5	<0.001***	0.34	0.007**	-0.33	0.98	-0.07	0.62
Wind	0.25	0.07	0.14	0.31	-0.05	0.57	0.03	0.47
Pollinators (bees)	0.1	0.29	0.15	0.26	0.05	0.4	0.05	0.4
Partial Mantel Test								
Fst, wind, IBD	-0.09	0.7	-0.03	0.56	0.14	0.3	0.09	0.38
Pollinators, wind, IBD	-0.02	0.52	0.23	0.15	0.01	0.47	0.06	0.4
Mantel Jost D								
IBD	0.53	0.001***	0.45	0.002**	-0.24	0.93	0.05	0.5
Wind	0.22	0.08	0.19	0.25	-0.05	0.56	0.16	0.27
Pollinators (bees)	0.03	0.43	0.12	0.27	0.09	0.34	0.12	0.28
Partial Mantel Test								
Fst, wind, IBD	-0.17	0.84	-0.06	0.62	0.09	0.36	0.16	0.29
Pollinators, wind, IBD	-0.12	0.75	0.24	0.12	0.06	0.39	0.11	0.3

3.5 Discussion

The aim of the present study was to evaluate the relationship between reproductive systems and dispersal mechanisms on genetic connectivity of wetland plant-species occurring in a meta-community system. In addition, we evaluated how landscape factors such as patch size and degree of isolation, as well as local plant-species richness affect population's genetic diversity. Our results show that both reproductive strategies and dispersal mechanisms have an effect on genetic diversity and genetic divergence, but with a larger effect of the reproductive system, specifically the degree of clonality. Clonality and the degree of outcrossing were correlated with genetic diversity: effective number of alleles (ENA), gametic richness (R), observed heterozygosity (H_O) and expected heterozygosity (H_E) within populations and species, while dispersal mechanisms were related to genetic divergence (F_{ST}) and therefore to connectivity. Genetic diversity was highest in the mainly outcrossing, insect-pollinated species, while higher genetic divergence and lower connectivity was found in insect pollinated compared with wind-pollinated species. Also, at a small local scale the inbreeding coefficient of the insect pollinated outbreeding species was positively correlated with kettle-hole area and surrounding plant-species richness, indicating obstacles to gene flow mediated by insects in a heterogeneous (micro) landscape.

TABLE 3.6: **Summary of the Analysis of Molecular Variance (AMOVA) based on 13, 15, 10 and 10 nuclear microsatellites among populations of *Oenanthe aquatica (Oa)*, *Lycopus europaeus (Le)*, *Typha latifolia (Tl)*, and *Phragmites australis (Pa)*, respectively.** Analysis of Molecular Variance among and within populations and global Fixation Index F_{ST} are shown. Significance levels are indicated with asterisks (***P< 0.001). To account for the polyploid nature of *P. australis*, an AMOVA was also performed based on presence/absence (binary) data.

Sp	Source of variation	df	Sum of squares	Variance components	Percentage of variation	F_{ST}
	Microsatellites data					
Oa	Among populations	11	383.9	0.7	14.3	0.14***
	Among individuals within populations	260	1130.2	0.3	6	
	Within individuals	272	1029	3.8	79.8	
	Total	543	2543.1	4.7	100	
Le	Among populations	7	506.9	2	37	0.37***
	Among individuals within populations	131	531.4	0.6	10.6	
	Within individuals	139	401.5	2.9	52.4	
	Total	277	1439.9	5.5	100	
Tl	Among populations	7	198.4	0.7	39.5	0.39***
	Among individuals within populations	146	150.1	-0.1	-4.1	
	Within individuals	154	181.5	1.2	64.6	
	Total	307	530	1.8	100	
	Binary data					
Oa	Among populations	11	762.5	2.6	20.5	0.15***
	Within populations	260	2647	10.2	79.5	
	Total	543	3409.5	12.8		
Le	Among populations	7	856.9	6.9	49.4	0.49***
	Within populations	131	925.2	7.1	50.6	
	Total	277	1782.1	14		
Tl	Among populations	7	382.1	2.8	59.4	0.59***
	Within populations	146	277.7	1.9	40.6	
	Total	307	659.8	4.7		
Pa	Among populations	7	1002.7	6	63.8	a 0.64***
	Within populations	179	609.5	3.4	36.2	
	Total	186	1612.2	9.4		

3.5.1 Effect of clonal growth

Genetic diversity related to clonal reproduction and breeding systems

Detection of genetically identical individuals suggests that these species can reproduce clonally. The low percentage of clonal individuals (2%) in *Oenanthe aquatica* suggests that this species is reproducing mainly sexually. This is supported by the data on genetic diversity, F_{IS} and Multilocus Linkage Disequilibrium (Table 3.3): genetic diversity was distinctly higher in *Oenanthe aquatica* (H_O= 0.5 ± 0.04, H_E= 0.6 ± 0.04) compared to the other three species (*Lycopus* H_O= 0.4 ± 0.07, H_E= 0.4 ± 0.05, *Typha* H_O= 0.2 ± 0.1, H_E= 0.2 ± 0.08, *Phragmites* H_O= 0.4 ± 0.02, H_E= 0.4 ± 0.2) (Figure 3.2). A high genetic diversity in mainly sexually reproducing *Oenanthe* compared to the selfing/clonal *Lycopus* was previously assessed in French populations with the same microsatellites: H_O: 0.48 H_E: 0.55 for *Oenanthe* and H_O: 0.4 H_E: 0.55 for *Lycopus* (Favre-Bac *et al.* 2016). In contrast, a relatively low genetic diversity (H_E: 0.22, H_O: 0.24) was found in the clonal species *Typha latifolia*, similar to populations occurring throughout Europe H_E: 0.38, H_O: 0.17 (Ciotir *et al.* 2017), North America H_E: 0.3, H_O: 0.21 (Kirk *et al.* 2011), though a bit higher than observed in populations from China H_E: 0.10, H_O: 0.07 (Zhou *et al.* 2016). Microsatellites variation in *Phragmites australis* has shown that lineages from populations in North America present high heterozygosity compared to native European ones (Saltonstall 2003).

Although *Oenanthe aquatica* was previously classified as self-compatible with a mixed breeding systems where outcrossing and self-fertilization are equally common (Kühn *et al.* 2004), our results showed a high genetic diversity, suggesting sufficiently frequent outcrossing as was also concluded by Favre-Bac *et al.* (2016) in populations in France. Additionally, we rarely detected selfing and/or clonal growth (lack of Multilocus Linkage Disequilibrium and $F_{IS} \sim 0$; Table 3.1, Table 3.3). However, significantly positive F_{IS} in this species, might be indicative of substructure within the pond (Table 3.3), due to a more likely outcrossing among closer located individuals. Lowest F_{IS} was found in *Typha* (F_{IS} = -0.102), but both, significant positive and negative values were found in 3 and 4 populations respectively (Table 3.3). These values could be explained by a combination of a high degree of clonality and dichogamous flowers in *Typha*. Positive F_{IS} could be attributed to selfing within flower or geitonogamous pollination between ramets as it was found in alpine clonal tussocks plants (Ning *et al.* 2018). It has been reported that in many clonal perennial species including *Typha*, *Scirpus* and *Sparganium*, a synchronization of sexual function among ramets occurs, possibly limiting inter-ramet geitonogamy (Cruden 1988) and facilitating outcrossing, i.e., reproduction among different genets. But at the same time, heterozygous clones that only reproduce clonally, retain the observed heterozygosity causing negative F_{IS} values. Another possible explanation of negative F_{IS} values may be somatic mutations generated by clonal growth without any adaptive value, as it was found in *Carex limosa* (Schwarzer 2018), which might be higher than in *Typha*.

Similarly, positive F_{IS} found in *Lycopus* (F_{IS} = 0.138) is likely related to geitonogamous selfing within ramets and/or genets. Still, the higher observed heterozygosity and gametic richness in *Lycopus* compared to *Typha* suggests that sexual reproduction is common in *Lycopus* and genetic variation probably mainly depends on pollination while *Typha* either only reproduces clonally or can self-fertilize. This is consistent with their breeding systems: *Lycopus* is a bee pollinated, facultatively xenogamous and self-compatible species whose production of fruits mainly results from outcrossing events and self-fertilization occurs rarely (Kühn *et al.* 2004); in contrast, *Typha* is a facultative autogamous with common self-fertilization and only rare outcrossing events (Kühn *et al.* 2004).

In *Phragmites*, genetic diversity (ENA, H_O, H_E) was similar to *Lycopus*, whereas gametic richness was similar to *Typha* (Figure 3.2A-D) suggesting that clonal growth in *Phragmites* has only little effects on genetic diversity, at least not as much as in *Typha*. Even though *Phragmites australis* was previously classified a self-incompatible species with an obligate xenogamous reproductive mode (Kühn *et al.* 2004), Lambert and Casagrande (2007) demonstrated that self-pollination can also occur. In addition, as in *Lycopus*, mean F_{IS} of 0.062 in 5 populations in *Phragmites* also suggests a deficit of heterozygotes and probable self-fertilization within the same ramet. However, this should be taken carefully due to the lack of significant test for F_{IS}.

Genetic diversity and F_{IS} related with landscape factors

The inbreeding coefficient was positively related to patch size (log area) in all species but not significant for *Phragmites* (Table 3.4, Figure 3.3A). This relationship suggests a substructure within the populations (small scale IBD) and/or may be caused by lower pollinator′ visitation rates. In *Lycopus*, *Typha* and *Phragmites*, the relationship between area and F_{IS} shows a high variation compared with *Oenanthe* (Figure 3.3A). The F_{IS} may be related to local clonal variability, rather than mere habitat size, in the former three species. Even though outcrossing events also occur in *Typha* and *Lycopus* as previously discussed, some populations in *Typha* showed clear signs of sustained clonality (negative F_{IS} and high r_d) while others show clear inbreeding (positive F_{IS}), probably caused by geitonogamous selfing. In larger patches, there may be several clones present and clonal individuals mate more likely with closely related individual ramets causing a substructure of the populations resulting in positive F_{IS}. For *Typha* and *Phragmites*, this scenario is likely due to their distribution in big patches (Figure S8). In the insect pollinated species *Lycopus*, a (counterintuitive) increase of F_{IS} with increasing patch size (Figure 3.3A), which was not related to population size as for *Typha* and *Phragmites* (Figure B8), might be better explained by its ability to self under lack of pollinators. *Lycopus* populations are very small and usually located in the middle of the kettle hole, always surrounded by bigger plant species (personal observation; Figure B8). Decreasing H_O with decreasing isolation might be related to a lower probability of pollinators (or seed dispersers) to find the plant surrounded e.g., by tall *Typha* and *Phragmites* populations. As a response to a lack of pollinators (or seed dispersers), self-pollination might occur in combination with clonal growth increasing homozygosity (Figure B6B).

Surprisingly, F_{IS} was significant negatively correlated with bee diversity in the wind dispersed species *Typha* and *Phragmites*, showing a decrease in F_{IS} with more species of bees. As these two species are wind dispersed, these relationships might reflect some correlated pattern, such as habitat heterogeneity (Figure 3.3). Those kettle holes harbouring larger number of bee species might constitute attractive patches for the bees to forage or to live, offering them a suitable environment.

3.5.2 Effect of dispersal

Trade-offs of being obligate outcrossing

Our results suggest that *Oenanthe aquatica* that possessed the highest genetic diversity and gametic richness (Figure 3.3A-D) is regularly outcrossing and an effective seed disperser, further affected by several biotic and abiotic landscape factors (Table 3.4). A strong negative relationship between plant-species richness and genetic diversity (H_O and H_E) and a positive relationship with F_{IS} suggest that this species is negatively affected by the number of plant species occurring in the pond (Table 3.4, Figure 3.3). This might be related to increased competition and decreased patch availability for seedling establishment. Furthermore, a higher number of plant species might constitute a higher competition for pollinators (see above).

Animal vs. wind dispersal vectors affecting gene flow

No Isolation-by-distance (IBD) relationship in *Typha* and *Phragmites* suggests no dispersal limitation, and therefore higher gene flow among populations also evidenced by the lower Delta K found for these species (both K= 2), compared with the other two species (*Lycopus*: K= 8, *Oenanthe*: K= 7), even within this relatively small mean area of 0.2 ± 0.1 ha (mean ± SD). These results suggest that dispersal mechanisms play a role in genetic divergence and therefore connectivity, where species that are adapted to pollen and seed transport by wind seems to have a higher gene flow compared with the insect-pollinated hydrochorous ones.

We did not detect any relationship between genetic distance and wind-speed or direction. However, in our system wind still might connect populations efficiently due to the small geographic distance between the kettle holes (0.2 – 15 km). Hence the reported inefficiency of wind as an effective driver for dispersal that decreases rapidly with increasing distance between populations (Whitehead 1969; Regal 1982) and that has a much shorter time longevity for wind-borne pollen compared with insect-pollinated species (Dafni and Firmage 2000) might not play a role in our landscape setting. Spatial autocorrelation analyzes showed spatial structure for all species (Figure 3.4). This seems to support IBD for all species, albeit to varying degrees; with a smoother decrease in the outcrossing *Oenanthe* compared with the selfing/clonal species. This is supported by the significant genetic divergence in all species measured by F_{ST} (Tables B3-B6) and an increase of global F_{ST} from outcrossing to selfing/clonal to mainly clonal species (Table 3.6), as expected (Table 3.1).

Seed dispersal in *Lycopus* and *Oenanthe* is mainly hydrochorous (Kühn *et al.* 2004; Moon and Hong 2006) which might restrict dispersal to other kettle holes through animal vectors, compared with the wind adapted pollen and seeds from *Typha* and *Phragmites*. Nevertheless, it is important also to account for dispersal by birds such as waterfowl (Charalambidou *et al.* 2005; Brochet *et al.* 2009; Soons *et al.* 2016). Previous studies have shown that *Phragmites australis* and *Typha latifolia* can be dispersed on the feathers of ducks (Brochet *et al.* 2009), seeds of *Oenanthe aquatica* on the feathers of water birds (Hroudova *et al.* 1992) and nutlets of *Lycopus europaeus* can be dispersed through endozoochory by mallards (Kleyheeg *et al.* 2015). In addition, it is also known that the wind direction does not constrain the flight of the birds (Green *et al.* 2008; Boonstra *et al.* 2017), which might partially explain the lack of relationship between genetic distance with speed and direction of the wind. However, to disentangle the real effect of dispersal vectors in population connectivity more experiments are needed in the future.

In addition to the clear pattern in gene-flow between wind vs. insect-pollinated species, the AMOVA results suggest that gene flow among populations is also well explained by breeding systems and degree of clonality. Lower variation among populations in the outcrossing and hardly clonal species *Oenanthe* suggests less population differentiation and more genetic exchange compared to the selfing-clonal species, which is supported by global F_{ST} measurements (Table 3.6). Furthermore, in *Oenanthe*, *Lycopus* and *Typha*, most of the genetic variation was explained within individuals (Table 3.6 microsatellite data) which might refer to outcrossing and geitonogamous (selfing) events for *Typha* and *Lycopus* as previously discussed.

3.6 Conclusion

We demonstrated that breeding and reproduction systems, from outcrossing to selfing and clonality are the main factors shaping genetic diversity and genetic divergence in metapopulations of wetland species occurring in an intensive agricultural landscape. We also found differences in dispersal mechanisms to play a role in influencing gene flow and connectivity. Additionally, we found that landscape factors, i.e., plant-species richness and the area of the wetland habitat were negatively correlated with genetic diversity in the outcrossing insect-pollinated species *Oenanthe aquatica*.

Clonal growth occurred in all species but to different degrees. The insect-pollinated species *Oenanthe aquatica* is a mainly outcrossing species that rarely forms clones. In the insect-pollinated *Lycopus europaeus*, outcrossing events within the same individual (geitonogamy) seems to be the common reproduction mode, although sporadically clonal growth might also occur. The wind dispersed species *Typha latifolia* and *Phragmites australis*, reproduce mainly clonally but also show patterns that indicate eventual sexual reproduction. In addition to clonality and breeding system, dispersal mechanisms seemed to affect genetic divergence and connectivity between populations with a higher gene flow in the wind dispersed species compared with the hydrochorus insect pollinated ones.

3.7 Availability of data and material

The dataset of microsatellites supporting the conclusions of this article is available in the [ZALF] repository, [unique persistent identifier and hyperlink to dataset(s) in http format will be soon available].

3.8 Funding

This work was supported by Deutsche Forschungsgemeinschaft (DFG), in the framework of the BioMove Research training group (DFG-GRK 2118/1).

3.9 Authors contribution

SLG and JJ designed the study. SLG collected the data in the field. SLG, RT, CS and JJ analyzed the data and discussed the results; SLG, RT, CS, RD and JJ wrote the paper, contributed critically to the drafts and gave final approval for publication.

3.10 Acknowledgements

We would like to thank Sandra Joensson for her help in the laboratory, Marco Donat for his interest resulted in his final master project about *Typha latifolia*; Frenze Geiger, Jeter Geiger, Carlos Landivar, Carlos Acame, Gabriela Onandia for their valuable help in the field, Benito Schoepke for plant identification, Wiebke Ullman for helping with the statistical analyses; Gernot Verch and Joerg Haase for providing wind data and to Gabrielle Schiro for providing the code to plot it. To Rok Cestnik for his help analysing the wind data and heterozygosity. We thank Florian Jeltsch for valuable comments and suggestions to the manuscript.

3.11 Supplementary Material

Table B1. List of plant and bee species occurring in the kettle holes.

Table B2. Details of primers and PCR for microsatellites analyses.

Table B3-B6. Pairwise matrices of F_{ST} of *Oenanthe aquatica, Lycopus europaeus, Typha latifolia* and *Phragmites australis*.

Table B7. Weighted matrix for the 20 selected kettle holes based on speed and direction of wind.

Table B8. Similarity matrix based on Jaccard-Index of presence-absence data of bee species.

Figure B1. Comparison of Multilocus Linkage Disequilibrium from microsatellite and binary datasets.

Figure B2-B5. STRUCTURE results showing barplots and most probable K groups in *Oenanthe aquatica, Lycopus europaeus, Typha latifolia* and *Phragmites australis*.

Figure B6-B7. Linear models representing the relationship between observed heterozygosity (H_O) and between expected heterozygosity (H_E) with the landscape biotic and abiotic factors: Patch size (A), isolation degree (B), plant (C) and bee richness (D).

Figure B8. Aerial photograph of one kettle hole showing the population size of the species.

CHAPTER 4

Wild-bee diversity in natural habitat islands in agricultural landscapes

Bumblebee on Salix spp. close to Falkenhagen, Uckermark.

Title	Intensive land-use of a surrounding agricultural matrix restricts wild-bees to natural island habitats
Authors	Sissi Lozada-Gobilard, Carlos Landivar Albis, Karolin Rupik, Marlene Patzig, Sebastian Hausmann, Ralph Tiedemann and Jasmin Joshi.
Journal	Landscape Ecology
Date of submission	Submitted 23th September 2019
Status	Under review LAND-D-19-00372.
Keywords	Wild-bees diversity, kettle holes, agricultural landscapes, wetlands, pollination services, biodiversity.

4.1 Abstract

The decline of wild bees worldwide due to land-use intensification, chemical as well as light pollution, habitat loss, invasive species and diseases endangers the pollination services they offer. Pollination is essential for plant population′ connectivity and ultimately for the functioning of the entire ecosystem. In this study, we assessed wild bee diversity in natural wetland habitats (kettle holes) embedded in an intensively managed agricultural landscape in northeastern Germany. We used color traps to sample individuals in 36 kettle holes and identified a total of 77 wild bee species. We hypothesized that habitat quality within kettle holes (i.e. flower resources) as well as habitat heterogeneity have a positive effect on bee diversity based on the assumption that different habitats provide a greater variety of nesting and food opportunities. Consequently, bee species with distinct life-history traits concerning sociality, feeding or nesting are expected to profit from habitat heterogeneity. We expected larger and less isolated kettle holes to harbor a higher bee species richness. In addition, we analyzed whether large-sized bees are less affected by the degree of isolation than smaller bees under the assumption that they can fly longer distances. In the kettle holes, we mainly found generalist feeding species, belowground breeding species and small solitary bees (9.8 ± 4.4, 8.9 ± 4.0, and 6.9 ± 2.9 species per kettle hole, respectively). Our results showed that an interaction between patch size and degree of isolation affected bee diversity. Bee-species richness was enhanced in small kettle holes if there were a relatively small number of 10 neighboring kettle holes within a radius of one kilometer restricting the potential for bees to forage outside their small island kettle hole patch. Local habitat quality — mainly related to flower resources — had a positive effect on bee diversity. Land-use configuration, i.e. the number of different landscape elements surrounding kettle holes (100–300m) was negatively correlated with local wild-bee diversity with urban cover as the only significant negatively correlated biotope. Hence, bee-species richness and Shannon diversity per patch decreased with surrounding urban cover. In contrast to our expectations, we found large bee size (characterized mainly by social bumblebees) to be affected by the degree of isolation. Our results highlight the importance of habitat quality within and surrounding the kettle holes to sustain wild bee diversity in this intensive agricultural landscape. We argue that habitat quality especially high flower resources and the availability of nesting sites are crucial for wild bee diversity in island kettle hole habitats. In addition, large sized bees are

depending on dense network of habitats/kettle holes.

4.2 Introduction

The decline of the entomofauna worldwide is mainly driven by habitat loss and conversion to intensive agriculture and urbanization (Sánchez-Bayo and Wyckhuys 2019). This decrease of insect species-richness and biomass is tied to a reduction of ecological services (e.g., pest control, pollination, dung burial, maintenance of diversity of higher trophic levels) and a consequent economic (Losey and Vaughan 2006) as well as cultural (Hector *et al.* 2001) loss. Among insects, bees are the world's dominant pollinators providing ecological and economic benefits to insect-pollinated wild plants and agricultural crops (Dicks *et al.* 2016; IPBES 2016). A recent meta-analysis on pollination services has shown that the functional diversity of bee species is positively correlated with crop yield of rapeseed fields (Woodcock *et al.* 2019). However, bee populations are vulnerable to landscape fragmentation and habitat degradation because they depend on floral resources and suitable habitats for nesting (Roulston and Goodell 2011). Studies have shown that the response of wild-bee populations to landscape configuration at local scale depends on life-history traits, particularly related to behavior, bee-body size, sociality and nest location (Bommarco *et al.* 2010; Gabriel *et al.* 2018; Kratschmer *et al.* 2019, but see Kennedy *et al.* 2013).

Habitats with sufficient floral resources and suitable for nesting and offspring provision are essential for wild-bee' foraging and survival (Tscharntke and Gathmann 2002). Wild-bee diversity depends on the size and degree of isolation of suitable patches: the smaller and more isolated the habitats, the lower the bee diversity they harbour (Steffan-Dewenter and Tscharntke 1999; Kremen *et al.* 2004). Consequently, a higher density of ideally large natural habitats near croplands enhances bee diversity (Kremen *et al.* 2004) securing successful pollination services and food production (Klein *et al.* 2003, 2007).

The northeastern German, intensive agricultural landscape is characterized by a high density of small water bodies, so-called kettle holes. These small ($<$ 2ha) water bodies were formed by retreating glaciers during the Ice Age (Kalettka *et al.* 2001). Within this landscape, kettles holes constitute hotspots of biodiversity acting as refuge for plant communities (Patzig *et al.* 2012; Lozada-Gobilard *et al.* 2019) and animal species (Céréghino *et al.* 2012). However, these natural landscape elements are threatened by structural degradation, drainage and pollution by pesticides (Kalettka *et al.* 2001; Céréghino *et al.* 2008).

Kettle holes form a meta-ecosystem of island-like aquatic habitats ideal to study meta-populations and meta-communities (Tiner 2003; Kalettka and Rudat 2006). Water-level fluctuations, high nutrient dynamics, and subsequent changes in space configuration shape the kettle-hole assemblies and the connectivity of communities (e.g., Onandia *et al.* 2018; Lozada-Gobilard *et al.* 2019). Even though, these wetland ecosystems might constitute suitable habitats for wild-bee populations and the pollination services they provide to the agricultural sector, the role of wetlands as habitats for pollinators has hardly been investigated (but see Vickruck *et al.* 2019).

In the present study, we analyzed whether kettle holes are suitable habitats to harbour wild-bee diversity. As bee species-richness and functional diversity was reported to have positive effects on pollination services, we evaluated the effect of landscape factors and habitat heterogeneity on wild-bee species- and functional trait diversity. Specifically, we analyzed the role of landscape factors: patch size and degree of isolation; and habitat heterogeneity on wild-bee diversity in island-like habitats under a meta-community framework to answer the following questions: 1) Do patch size and degree of isolation have an effect on wild-bee diversity? 2) How does land-use configuration (i.e., land-use around kettle-hole habitats) and environmental conditions around kettle holes (plant cover, hydroperiod) affect wild bee diversity? 3) Is bee body-size related to the degree of isolation? 4) Are bee functional groups (body size, sociality, feeding and nesting behavior) differentially affected by habitat heterogeneity?

We hypothesized that kettle holes are key habitats for wild-bee′ nesting and foraging within an intensively managed agricultural matrix. Considering the meta-community framework, we expected that the diversity of wild-bees increases with patch size and habitat heterogeneity and decreases with higher isolation. As different body sizes reflect the capability for distance flights to forage, we expected size classes of wild-bee species to be differentially affected by the degree of isolation. Based on the assumption that more heterogeneous habitats provide more niche opportunities in terms of food and nesting resources, or dispersal routes, we expected a positive relationship between habitat heterogeneity and bee diversity. Based on i) Ekroos *et al.* (2013) who have shown that social bees that form small colonies only move relatively small distances to forage in local suitable habitats, ii) Klein *et al.* (2003) who found that light intensity is a main factor driving solitary bees that nest on the ground and iii) Ngo *et al.* (2013) who reported that open areas can enhance below-ground nesting species, wild-bee species differing in sociality and nesting behaviour should show different responses to habitat heterogeneity.

4.3 Methods

4.3.1 Study area

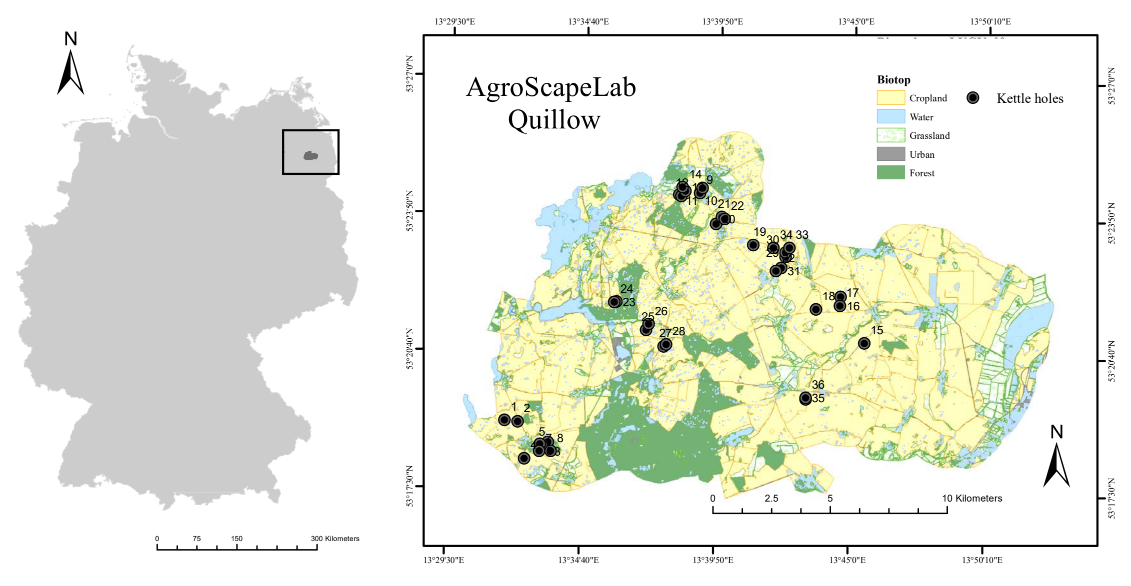
Our study area was located in the "AgroScapeLab Quillow", an agricultural landscape laboratory in the Quillow catchment area, which was established by the Leibniz Centre for Agricultural Landscape Research (ZALF) e.V. approx. 100km North of Berlin (Germany, Brandenburg). This area comprises c. 168 km^2 and provides a high density of small kettle holes with periodic to (semi)permanent water regimes (Pätzig *et al.* 2012, Figure 4.1). The predominant land use of this area is intensive agriculture with maize, wheat, and rapeseed as the main crops. The water regime of the kettle holes in this region is influenced by a subhumid climate with precipitation of 450–600 mm year-1 and potential evapotranspiration of 600–650 mm year^{-1} (Kalettka and Rudat 2006). Within this landscape, we randomly selected 36 kettle holes with an average patch size of this natural landscape element of 0.26 ha (Table 4.1, Figure 4.1).

4.3.2 Landscape parameters

Information on landscape composition and configuration such as type of land use in the area, topography, crop type surrounding the kettle hole, the presence and cover of trees and hydromorphological type of kettle holes according to the classification of Kalettka and Rudat (2006) were taken from a ZALF database (biodiversity monitoring data of the kettle holes by T. Kalettka at Leibniz-Zentrum für Agrarlandschaftsforschung, Müncheberg Germany). In addition, we calculated area and the degree of isolation measured as the distance to the closest neighbor and number of neighbors at different radii from 50–1000 m using ArcGIS 10 (ESRI 2011) based on ZALF official maps of the sites surveyed in 2016.

4.3.3 Wild-bees collection and identification

To assess the bee diversity we used the pan trap method classified as the most efficient and cost-effective in agricultural and seminatural habitats (Westphal *et al.* 2008). In each kettle hole, we positioned four sets of colour traps each consisting of a pair of red, blue and white pans. All pans were 27 cm in diameter 5 cm high, and were sprayed with UV-reflecting colour (Figure S1). The traps were filled with water and 3–4 drops of dishwashing detergent. We left the traps 48 hours before emptying and kept the samples in 70% EtOH. All collected bees and bumblebees were dried and pinned. Identification of specimens was performed by K. Rupik (University of Bielefeld) and C. Sauer (Museum für Naturkunde, Berlin) following established taxonomic keys for Germany and Europe.



Kette holes

1. Boi 28
2. Boi 40
3. Buc 1338
4. Buc 1590
5. Buc 1598
6. Buc 1599
7. Buc 1604
8. Buc 2565
9. Dam 892
10. Dam 893
11. Dam 907
12. Dam 908
13. Dam 910
14. Dam 911
15. Fal 149
16. Fal 183
17. Fal 187
18. Fal 190
19. Kra 312
20. Kra 805
21. Kra 807
22. Kra 808
23. Par 1228
24. Par 1229
25. Raa 1189
26. Raa 2489
27. Raa 606
28. Raa 607
29. Rit 258
30. Rit 259
31. Rit 265
32. Rit 269
33. Rit 275
34. Rit 287
35. Wil 235
36. Wil 236

Fig. 4.1. Study area Agricultural Landscape Laboratory "AgroScapeLab Quillow" in the Quillow catchment area located in north-eastern Germany (Brandenburg). This agricultural landscape is characterized by a high density of kettle holes. Black points denote our selected kettle holes N=36. Percentage of land use in the area is 65% cropland, 17% forest, 9% grassland, 5% water, and 4% urban.

4.3.4 Floral traits

For analyses of bee-body size and isolation effects of the kettle holes, we classified our species into three groups: Small, Medium and Large, which correspond to foraging distances of Small=100–300m, Medium= 300–600m, Large=600–1200m based on Greenleaf *et al.* (2007; Table S1). Additionally, traits corresponding to behaviour and life style (sociality; see below), location where they have their nest (nesting) and degree of specialization for nectar plants (lecty) were used to further classify them. Sociality: solitary, eusocial and parasites; nesting location: above and belowground; and lecty: polylecty and oligolecty. Definitions of these traits are provided in Table S5.

4.3.5 Vegetation mapping and classification

Plant-species richness and percentage cover of vascular plants was assessed of the entire kettle-hole area, accessing the inundated area with chest waders. Plant-species cover was determined in 1–5% percentage steps *in situ* and converted into the 14-part Londo scale (Londo 1976). Due to a large number of species-cover entries with one percent or less, we included an additional class "0.1" for coverage values below 1% and assigned the 1% plant-species coverage to the Londo scale "1" (Table S2). This was necessary to avoid an overestimation of total vegetation cover by rare species. The dominant vegetation type of each kettle holes was determined following Kalettka and Rudat (unpublished) based on the work of Hamel (1988) and Luthardt and Dreger (1996). Unfortunately, due to logistic problems, vegetation cover was only possible to assess in 28/36 kettle holes (Table S3).

4.3.6 Floral cover

During the sampling of the bee specimens, we recorded the phenology of the most abundant insect-pollinated plant species around the kettle holes and estimated the flower-resource availability by four classes: "none", "very low", "low" and "medium".

TABLE 4.1: **Summary table of size (area), degree of isolation (number of neighbors within a 200 and 1000 m radius) and wild-bee diversity alpha, beta gamma.** Mean number of number of individuals (abundance) and number of species (species richness) occurring in the kettle holes. Total number of wild-bee species (gamma diversity) excluding *Apis melifera* and abundance. Beta diversity analyzes on wild-bee community includes species turnover, nestedness and overall beta diversity based on site dissimilarity (Jaccard dissimilarity).

Variable	Description	Mean ± SD	Min	Max
Patch size	Area [ha]	0.26 ± 0.19	327	7239
Degree of isolation	Neighbor kettle holes (200 m)	1.1 ± 0.6	0	2
	Neighbor kettle holes (1000 m)	48.1 ± 18.4	9	77
Bee diversity	Abundance	37.8 ± 36.6	6	161
	Shannon-Index	2.0 ± 0.4	0.7	2.5
	Species richness (alpha diversity)	11.2 ± 4.6	2	25
		Turnover	**Nestedness**	**Overall**
	Beta diversity	0.944	0.021	0.965
	Gamma diversity (total)	—	—	80

4.3.7 Statistical analysis

We characterised wild-bee diversity i) as total number of species detected in each kettle hole (alpha diversity), ii) total regional diversity (gamma diversity) and iii) the species turnover ratio between regional and local species diversity (beta diversity) based on the Jaccard similarity index. Additionally, iv) we counted the abundance of individuals and v) calculated the Shannon Diversity Index [H′]. To evaluate the sampling size in terms of number of kettle holes, we calculated a rarefaction curve based on 9999 permutations. A rarefaction curve is constructed by repeatedly re-sampling the pool of N samples (or N individuals), at random, plotting the average number of species represented by 1, 2,. . . N samples or individuals (Gotelli and Colwell 2001).

We tested if species richness of bees depends on kettle-hole area (patch size) and / or on connectivity, i.e. on the number of kettle holes within a radius of 500 m (degree of isolation). For this, we previously tested which buffer radius (20, 50, 100, 200, 500, 1000 and 2000 m radii) influenced bee-species richness and selected the most parsimonious model based on AIC using the "multifit" function in R. Due to very low number of ponds within small radii, we discarded the first two buffers (20m, 50m). For radii between 50–2000m, we estimated habitat heterogeneity represented by the SHDI (Shannon Habitat Diversity Index; Eiden *et al.* 2000) using the map for biotopes and land-use of Brandenburg (2013) corresponding to the study area. Since we did not get any difference between the 1000–2000m radii, we only report SHDI measurements from 50–1000m.

Landscape diversity and configuration was characterized by calculating the Shannon Habitat Diversity Index (SHDI) in combination with vegetation cover in the kettle. SHDI was based on the relative cover (area) of eight different biotopes (cropland, forest, grassland, water, urban, urban green open spaces, hedges and bogs) at different radii from 50–1000 m around the ponds. In addition, each biotope was analyzed individually: i) *Croplands* refer to cultivars of maize, cereals and canola, ii) *forest* include natural and managed ones, iii) *grasslands* include meadows and pastures, iv) *water* include ponds, streams and lakes; while v) *bogs* refer to peatlands, vi) *urban* refers to sealed roads and buildings, and vii) *urban green* open spaces refer to gardens, parks and finally viii) *hedges* including shrubs and trees in natural corridors between fields and artificial corridors in avenues. All calculations were performed using a biotope map of the study area in R version 3.5.2.

We further evaluated habitat quality based on the dominant vegetation type, ad well as hydromorphology and hydroperiod of the kettle holes. We compared wild-bee diversity (species richness and Shannon-Index) according to the type of kettle hole (Table 4.2) excluding "Open" types due to low number of kettle holes belonging to this category (n=2). We also compared wild-bee diversity according vegetation cover (%) and flower-cover categories (none, very low, low and medium). Additionally, we tested whether the surrounding type of cropland (cereal, maize, canola), including grassland had an effect on bee diversity.

TABLE 4.2: **Characterization of types of kettle holes according to the dominant vegetation and their corresponding plant and bee diversity.** This classification of kettle holes is based on dominant vegetation (vascular plants) calculated using Deck Londo coverages in combination with geomorphology and hydroperiod (see methods for details). Plant and bee diversity is based on species richness (S) and Shannon-Index (H'). Data is shown in mean ± SD and N represents the number of kettle holes corresponding to each type.

Type of kettle hole	N	Plant diversity		Bee diversity	
		S	H′	S	H′
Edge reed	12	29.5 ± 7.9	1.6 ± 0.4	13.2 ± 4.7	2.1 ± 0.2
Edge wood	8	34.8 ± 9.5	1.6 ± 0.4	13.6 ± 4.0	2.2 ± 0.3
Full reed	10	21.6 ± 8.1	1.1 ± 0.6	7.9 ± 2.9	1.7 ± 0.5
Full wood	4	33.3 ± 8.7	1.2 ± 0.7	11.3 ± 4.1	2.1 ± 0.5
Open (puddle)	2	13.5 ±2.1	1.2 ± 0.1	5.5 ±0.7	1.7 ± 0.2
TOTAL	36	27.3 ±9.7	1.4 ±0.5	11.2 ± 4.6	2.0 ± 0.4

	Description of types of kettle holes
Edge reed	Open water body with presence of reed, canary reed grass and sedges.
Edge wood	Open water body with predominant ruderal vegetation or shore woods.
Full reed	No distinct water body with predominant reed, canary reed grass and sedges all over.
Full wood	No distinct water body with wood species dominant in the middle such as willow, birch or alder.
Open (puddle)	Low in plant species. Characterized by periodic tillage.

We used GLMs (Generalized Linear Models) and model selection based on the AIC criterion on a set of models with different combinations of vegetation cover, floral resource availability, and SHDI and their interactions to test their effect on bee diversity (i.e., bee-species richness, bee-species abundance as response variables). Species richness and abundance models were formulated as GLMs with Poisson distribution. Initially, region was used as predictor in every model to encompass region-specific effects, but it was later discarded since models where it was included did not perform better than without. Evaluation and visualization of relevant effects of the most parsimonious models were computed with the R package "effects" (Fox 2003).

To evaluate whether body size is related to degree of isolation, we performed correlation analyses between the different buffers (50–1000m) and the diversity (Shannon and species richness) of small, medium and large-sized wild-bees. To test if wild-bees had a different trait response to habitat heterogeneity, species richness and Shannon by sociality, nesting and lecty were analyzed separately using GLMs selection based on the AIC criterion. Finally, we compared bee functional richness by: sociality, lecty, nesting type and body size using the functions "anova" and "TukeyHSD" from the package stats in R (version 3.5.2). Definitions of bee functional traits are based on Kratschmer *et al.* (2019) and references therein (Table S5).

4.4 Results

We sampled a total of 1321 insect individuals corresponding to 77 species including *Apis mellifera*, plus one “cf. (conformis)” (cf. *Nomada minuscula*) and one undetermined Lasioglossum “spec.” (species list in Supplementary material: Table S1). According to the accumulation rarefaction curve (Figure S2), the sampling effort was reliable. The highest bee abundance was found in "Falkenhagen 187" with 161 individuals and the lowest abundance with 6 individuals in Buchenhain 1338 and Damerow 893, respectively (Table S3). In general, we detected a mean abundance of 37.8 ± 36.6 individuals per kettle hole. Mean Shannon-diversity was 2.0 ± 0.4 for all species with a maximum of 2.5 recorded in Parmen 1229 and minimum of 0.7 in Damerow 908 (Table 4.1, Table S3). Alpha diversity of wild-bees varied from three species in Damerow 908 up to 25 species found in Damerow 892 with a mean species richness per patch of 11.2 ± 4.6 (Table 4.1). Analysis of beta diversity showed a high dissimilarity (0.97) among communities, with turnover of species (0.94) as the main process compared with nestedness (0.02; Table 4.1).

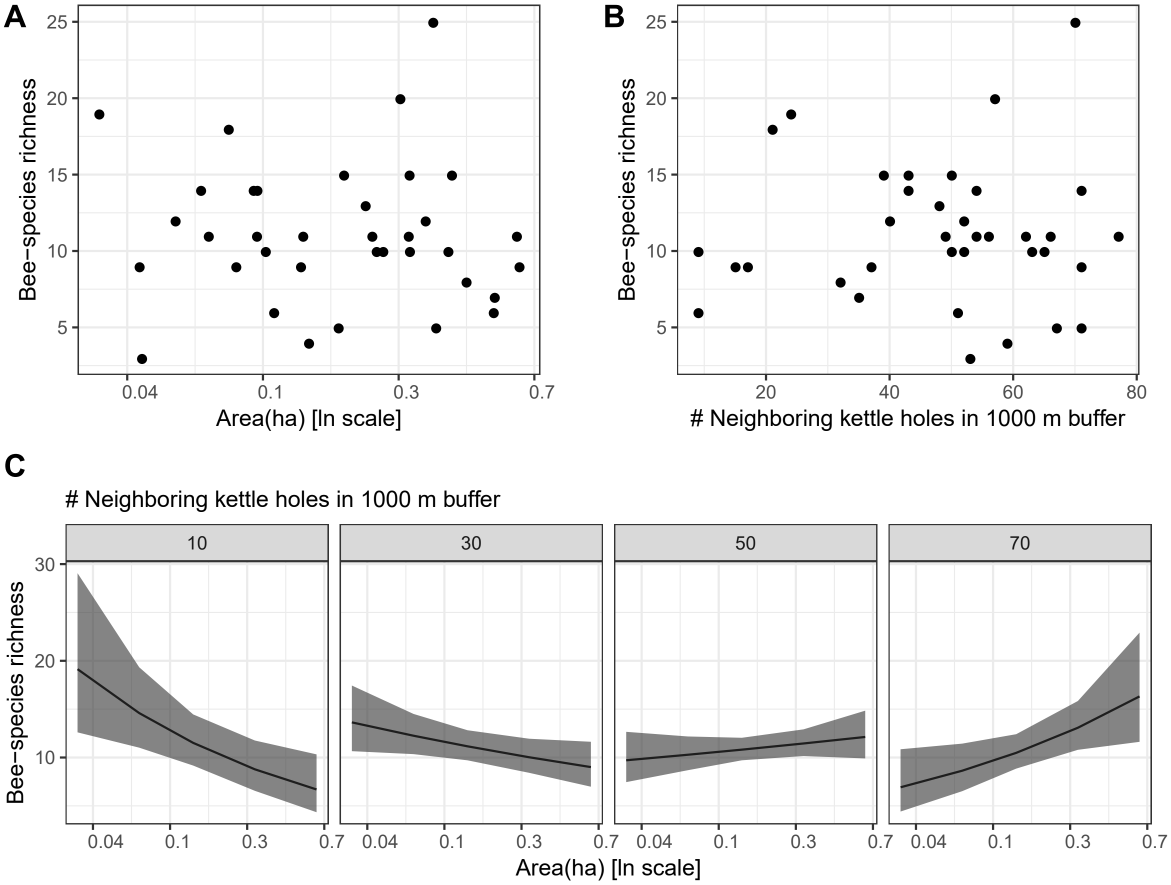


Fig. 4.2. Relationship between wild-bee species richness with patch size (area of the kettle hole in hectares), degree of isolation (number of neighbor kettle holes within a 1000m- radius) and their interaction. There was no relationship between species number of wild-bees and area of the kettle hole (A), nor with the degree of isolation (B), but the interaction between area and isolation showed an effect on species number (C).

Apis mellifera was present in 21/36 (58%) of the kettle holes. However, a total of 39 *Apis mellifera* individuals was sampled representing only 2.8% of the total community. *Andrena haemorrhoa* was the most abundant species with 237 individuals (17.2%), followed by *A. nigroaena* with 189 individuals (13.7%) and *Lasioglossum pauxilum* with 156 individuals (11.3%). In total, 29 bee species were represented by one individual only.

Neither the area of the kettle hole (patch size) nor the number of neighboring kettle holes (degree of isolation) at different buffers (100–2000m) had an effect on bee-species richness or abundance (Figure 4.2A–B) when tested for each buffer distance individually (all $p>0.05$). However, in a combined model, there were significant interaction effects: e.g., the number and area of kettle holes within a radius of 1000m had a significant effect on bee-species richness (S; Table S4, model 10 lowest AIC). At a 1000m-radius, bee-species richness was enhanced in small kettle holes if there were a relatively small number of 10 neighboring kettle holes within a radius of one kilometre, whereas bee-species richness got largest only in the large kettle holes if they were surrounded by 70 kettle holes in this radius (Figure 4.2C) indicating a threshold of minimum habitat size and habitat abundance with bee diversity in small kettle holes not benefitting from neighboring habitats. Details of the GLMs can be found in Table S4.

Habitat heterogeneity had a negative effect on bee species-richness (Figure 4.3A) and bee diversity (Table 4.3). To better understand why heterogeneity was negatively related with bee-species richness and diversity, the effect of each biotope was tested individually. We found a significant negative effect of urban (sealed space) on bee diversity between 125-300 m. Bee-species richness and Shannon diversity per patch decreased with surrounding urban cover (Table 4.3, Figure 4.3B).

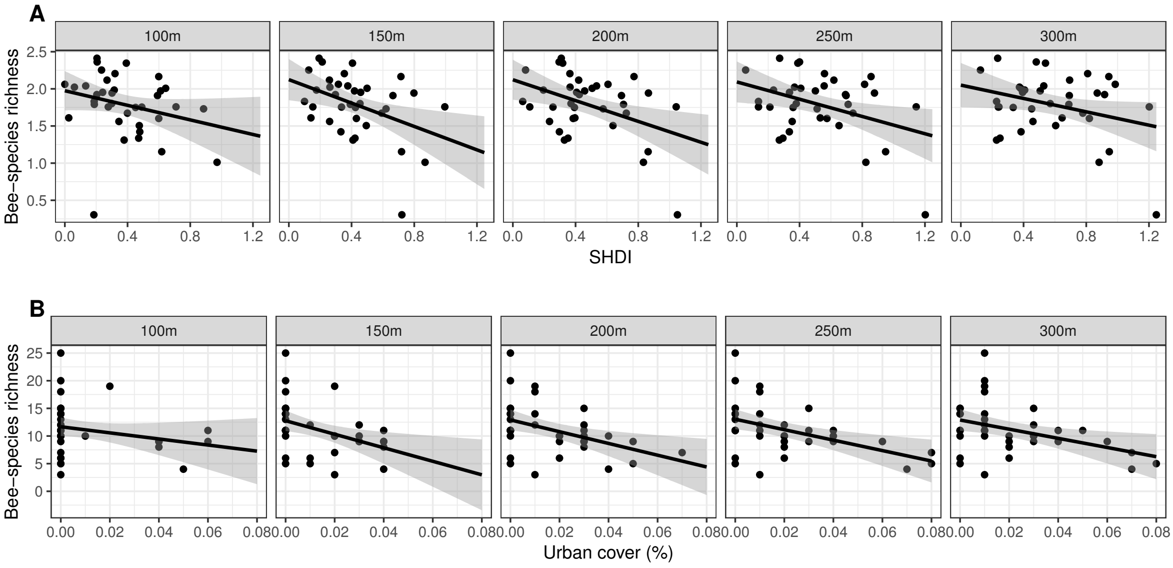


Fig. 4.3. Relationship between bee-species richness with Shannon Habitat Diversity Index (SHDI) and cover of cropland [%] at different radii (100–300 meters). Bee-species richness per patch decreased with landscape heterogeneity (A) and urban cover (B).

TABLE 4.3: **Summary of Generalized Linear Models GLMs to evaluate the effect of habitat heterogeneity on wild-bee species richness and Shannon-Index diversity.** Habitat heterogeneity was measured as SHDI (Shannon Habitat Diversity Index) based on the area of 8 different biotopes. Individual effect of these biotopes on wild-bee diversity is also shown. Most parsimonious model based on AIC criterion was selected for each biotope and the overall heterogeneity from a set of buffers from 50 up to 1000 m radii. Additional a range of radii of different buffers are shown for corresponding significant biotopes. Habitat heterogeneity has an effect on bee diversity between 100–300m; only the area of urban showed a significant negative effect on wild-bee species richness and Shannon diversity between 125–300m.Significance levels are indicated with asterisks: ***$P < 0.001$, ** $P < 0.01$, * $P < 0.05$

	Best buffer model	**AIC**	**Estimates**	**P value**	**Significant buffers**
Bee species-richness					
Heterogeneity SHDI	175 m	210.77	-8.27	0.012*	100–275m
Cropland	50 m	216.31	2.06	0.29	
Forest	1000 m	216.54	6.17	0.35	
Grassland	100 m	217.09	-1.89	0.52	
Water	300 m	216.5	-57.87	0.32	
Urban (streets and houses)	250 m	208.71	-94.46	0.004**	225–275m
Urban green open spaces	600 m	213.71	-176.23	0.18	
Hedges	525 m	214.5	125.16	0.09	
Fens and bogs	1000 m	214.34	-223.53	0.09	
Bee diversity [H′]					
Heterogeneity SHDI	150 m	35.43	-0.56	0.045*	125–225m
Cropland	25 m	37.73	0.21	0.171	
Forest	600 m	37.17	0.99	0.12	
Grassland	100 m	38.94	-0.22	0.39	
Water	300 m	37.17	-7.65	0.12	
Urban	250 m	33.93	-6.62	0.02*	125–300m
Urban green open spaces	25 m	37.26	-12.14	0.13	
Hedges	500 m	38.79	-6.54	0.35	
Boglands	25 m	38.69	-0.3	0.32	

To assess the importance of local habitat quality related to bee diversity within individual patches, we characterized the kettle holes into five groups based on dominant vegetation, geomorphology and hydro-period (Table 2). A 17% lower number of bee species (Figure 4.4A) and of bee diversity (Figure 4.4B) was found in the "Full reed type" kettle holes ($F_{(3,30)}$=4.12, p=0.01 and $F_{(3,30)}$=4.10, p=0.01, respectively). In contrast, neither bee-species richness nor diversity differed depending on the land use surrounding the kettle holes (including the eight types)(Figure S3; $F_{(3,30)}$=0.5, p=0.6; $F_{(3,30)}$=0.78, p=0.5 respectively). Wild-bee species number did not increase with plant cover (Pearson correlation: t=0.17, p=0.8) (Figure 4.5A); but increased with flower cover and therefore with floral resources ($F_{(3,32)}$=3.7, p=0.02): There was significantly higher number of bee species in patches with low and medium flower cover compared with those without or with very low flower cover (Figure 4.5B).

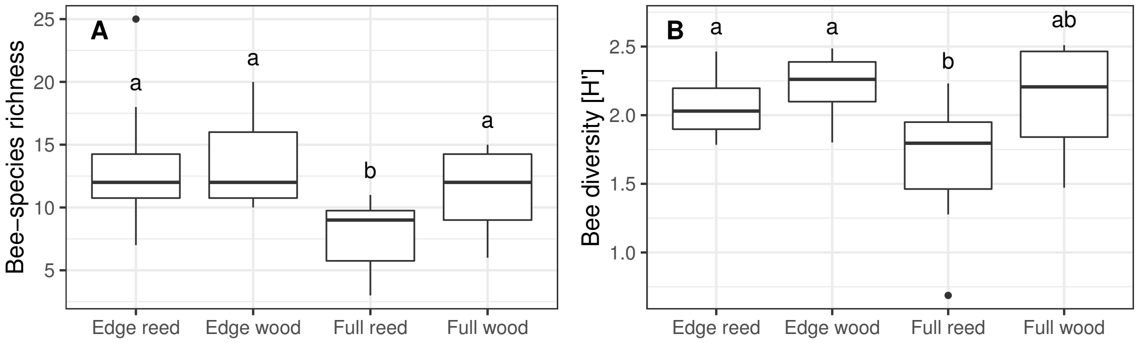


Fig. 4.4. Comparison of wild-bee species richness and diversity (H') according type of kettle hole based on dominant vegetation and type of cropland including grassland surrounding the kettle holes. Bee species richness was significantly lower in "Full reed" vegetation type (A), as well as Shannon-Index of bee diversity (B) compared to edge types and full type wood. Letters represent differences according post-hoc Tukey test.

Finally, we found differences in functional diversity of wild-bee species. From the total of 74 species found (excluding *Apis mellifera*, cf. *Nomada minuscula*) and *Lasioglossum spec.*) 37 (48.0%) possessed a small body size, followed by 25 (32.5%) of medium size and finally 12 species of large bees (15.6%). Most of the species were solitary 57.1% (44 species) followed by eusocial 22.0% (17 species) and finally parasitic bees 16.8% (13 species). Almost all species were polylectic 88.3% (68 species) and only 7.8% oligolectic (6 species). A total of 54 species build their nest belowground (70.1%) and 8 aboveground (10.4%). Mean number of species per kettle hole showed the same tendency for sociality, lecty and nesting type (Figure 4.6A,C, D), but not for body size (Figure 4.6B). This might be explained by the differences in proportion of individuals per category, with 63% medium, 31% small and 6% large-sized individuals. Small and medium species are mainly solitary with underground nests. Large bee species are all polylectic, most of them eusocial and with similar proportions of nesting above and underground (Figure S4).

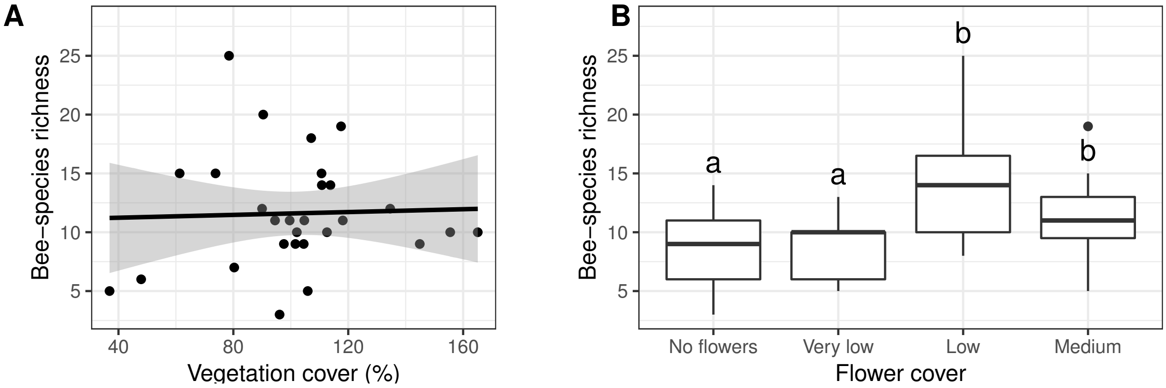


Fig. 4.5. Relationship of wild-bee species number with vegetation cover and flower cover within the kettle hole. Vegetation cover does not increase the number of wild-bee species in the kettle hole (A), but those with low and medium flower coverage, harbour a significant higher number of wild-bee species (B). Note that the vegetation cover is bigger than 100% due to a more than one layer of vegetation; mainly herb and trees. Letters represent differences according post-hoc Tukey test.

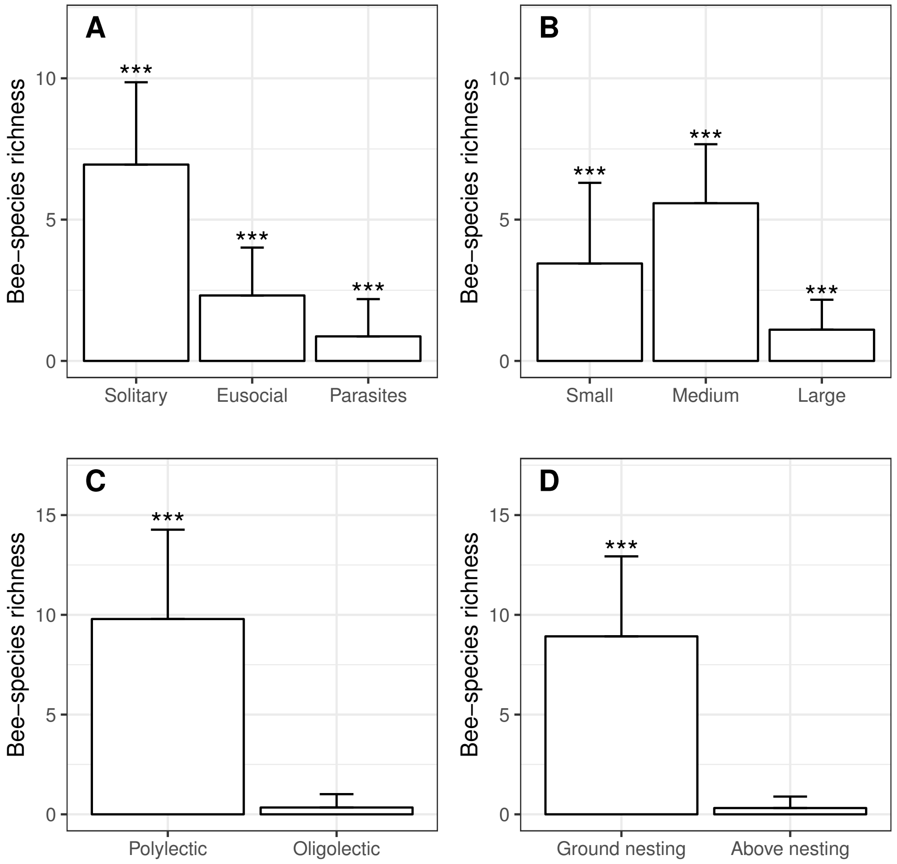


Fig. 4.6. Mean number of bee species according different functional traits: sociality (A), body size (B), Lecty (C) and nesting type (D). Differences in number of species were found for all functional traits. Bee species are mainly solitary, polylectic with a type of nesting under the ground and a medium body size. Body size is an approximation for foraging distances: Small = 100–300m, Medium = 300–600m, Large = 600–1200. Significance levels are indicated with asterisks: ***P< 0.001, **P<0.01, *P<0.05.

Habitat heterogeneity negatively affected bee-species richness and Shannon diversity regarding all traits mainly (but “oligolectic” and “aboveground”), with a significant effect between 150–275m. Parasitic bees were positively correlated with habitat heterogeneity at larger buffer zones (500–650m) (Table 4.4). The degree of isolation calculated as the total number of kettle holes at different buffer radii (100–2000) was positively correlated at a 1000m and, nearly significantly correlated at a 2000m buffer with the relative abundance of large individuals, (Pearson: t= 2.03, p=0.04, t= 1.7, p=0.09, respectively) (Figure 4.7) and species (t= 1.82, p=0.07, t= 2.3, p=0.02) (Figure S5) whereas the relative abundance of small and middle-sized individuals was not influenced by the degree of isolation.

4.5 Discussion

The main objective of this study was to assess the effect of environmental factors on wild-bee diversity in natural wetland habitats embedded in an intensive agricultural landscape. We assessed wild-bee diversity patterns and evaluated the effect of degree of isolation and patch size on wild-bee communities under a metacommunity framework, as well as the

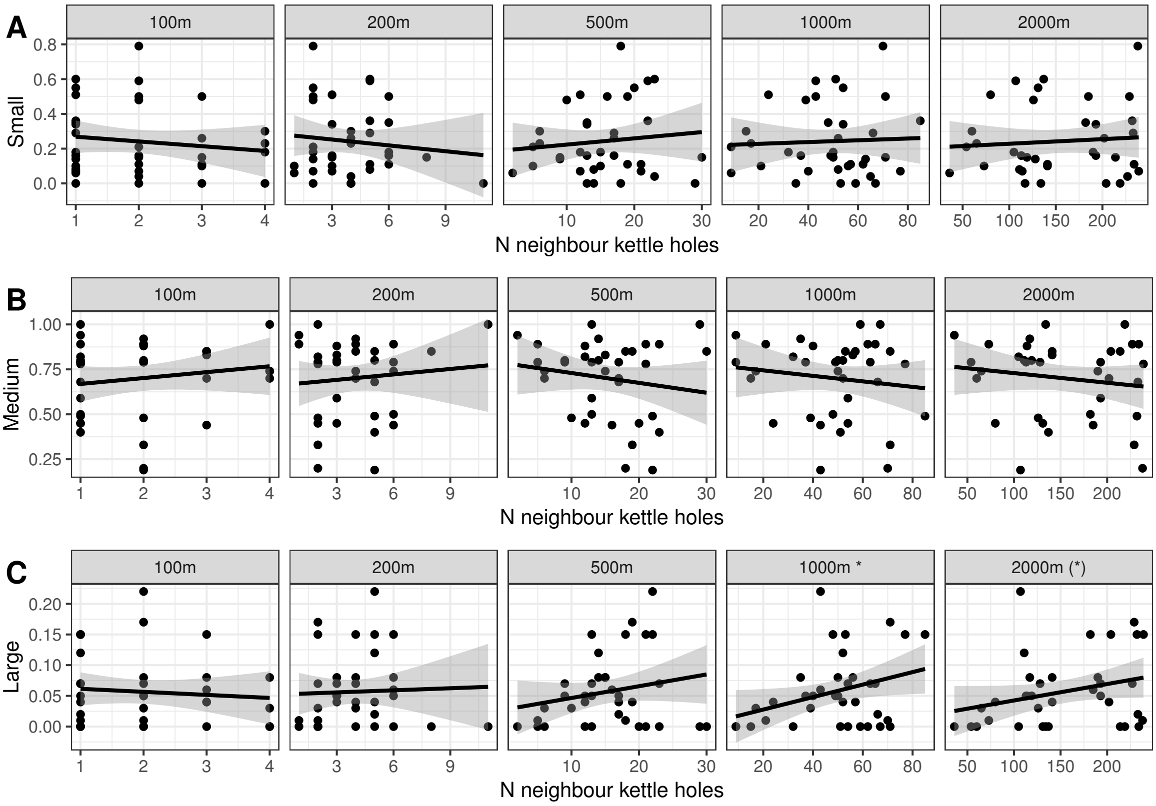


Fig. 4.7. Relationship between body sizes with degree of isolation. Relative abundance of individuals was calculated according to three groups: Small, medium and large. We found a positive correlation only at 1000 m and, to a lesser degree, at 2000 m for relative abundance of large individuals. Significance levels are indicated with asterisks: *P<0.05, (*) P=0.09.

effect of habitat heterogeneity and quality within and surrounding the kettle holes. Additionally, we evaluated the trait response of bee functional communities to isolation and heterogeneity. Our results show that these wetland habitats harbour a high diversity of wild-bees, they might serve as important habitats to forage, enhance connectivity among these meta-ecosystems and provide pollination services of the entire agroecosystem.

4.5.1 Diversity patterns

Our study is the first contribution assessing wild-bee diversity in this intensive agricultural landscape of northeast Germany. Considering our small study area ($\sim$200 km^2) and the restricted sampling time, we found a high gamma diversity of 77 species with a mean of 11.2 ± 4.6 species per kettle hole, compared with other ecosystems. For example, in vineyards of Spain, Austria, France and Rumania Kratschmer *et al.* (2019) found a total of 113 species (20–64 species/country), or 179 species within agricultural fields in western Europe (Hass *et al.* 2018). Settele *et al.* (2008) found 105 species of wild-bees in wet meadows in Poland, while Vickruck *et al.* (2019) found 132 species in potholes in Canada.

A parallel study in the same area assessed wild-bee diversity in grasslands using the same method but three times during the year (June, July, August) with a slightly higher total of species (82 vs. 77) (Bergholz, unpublished data). This suggest that a higher effort in space (4 traps per kettle hole) combined with a high number of sites, compensate the one-time

TABLE 4.4: **Summary of Generalized Linear Models GLMs to evaluate the different trait response of wild-bee species richness and Shannon-Index diversity effect on habitat heterogeneity.** Habitat heterogeneity was measured as SHDI (Shannon Habitat Diversity Index) based on the area of 8 different biotopes. Most parsimonious model based on AIC criterion was selected for the overall heterogeneity on functional trait groups from a set of buffers from 50 up to 1000 m radii. Habitat heterogeneity has a negative effect on bee richness and diversity regarding all traits mainly between 150–275m. Except for those species that are parasitic a significant positive effect of heterogeneity between 400–650m was found. Significance levels are indicated with asterisks: ***P< 0.001, ** P< 0.01, * P< 0.05

	Best buffer model	**AIC**	**Estimates**	**P value**	**Significant buffers**
Bee species richness					
Sociality					
Solitary	175m	175.81	-5.26	0.01*	125–275
Eusocial	200m	137.37	-2.8	0.01*	100–275
Parasitic	525m	122.75	1.54	0.025*	425–650
Lecty					
Polygolectic	175m	206.55	-7.32	0.018*	100–250
Oligolectic	200m	69.52	-0.77	0.063	
Nesting					
Aboveground	850m	65.54	0.4	0.18	
Below ground	175m	196.38	-7.94	0.004**	100–300
Bee diversity [H']					
Sociality					
Solitary	225m	34.55	-0.48	0.04*	150–250
Eusocial	200m	57.42	-0.69	0.049*	150–225
Parasitic	500m	49.68	0.5	0.045*	500–550
Lecty					
Polylectic	150m	39.12	-0.63	0.046*	125–200
Oligolectic	150m	-14.53	-0.19	0.2	
Nesting					
Aboveground	25m	-26.5	0.16	0.16	
Below ground	200m	36.76	-0.7	0.009**	125–275

restriction. Sampling reliability is supported by the rarefaction curve of species (Figure S2) showing that the number of sampled kettle holes (n=36) is very close to the maximum of species accumulation. In this sense, we can affirm that the colour trap method used demonstrated to be low cost and effective (Westphal *et al.* 2008) for these wetland habitats.

4.5.2 Kettle holes as shelters for pollinators

Wetland habitats constitute as hot spots for biodiversity in intensive agricultural landscapes (e. g. (Céréghino *et al.* 2012; Schirmel *et al.* 2016). Even though kettle holes are known to harbour high diversity, studies assessing wild-bee diversity in wetland habitats are rare. However, a recent study in Canada has demonstrated that pothole wetlands are important resources for native bees enhancing pollination for natural plant communities and crops such as canola (Vickruck *et al.* 2019).

From a total of 21 wild-bee species that were observed pollinating flowers of canola (*Brassica napus*), 19 were found in the kettle holes (Table S1 marked an *, plus *Anthopora plumipes* and *Bombus hypnorum*) (field observations Hausmann 2016). Although our study did not specifically focus on the comparison of wetland habitats with croplands, these observations give some insights about the importance of these ecosystems supporting wild-bee diversity and therefore pollination services in the croplands.

4.5.3 Patch size and isolation

Habitat fragmentation and isolation not only affect wild-bee species richness but also have a negative effect on plant-pollinators interactions, causing extinctions and degradations of the entire ecosystem (Aizen *et al.* 2016). Our results however, showed no effect of only the degree of isolation on wild bee diversity, but in combination with habitat area the number of neighbor kettle holes in a 1000m buffer showed an effect on bee-species richness (Figure 4.2, Table S4). When there was a low number of ponds within a 1000m radius number of bee species decreased with habitat area, whereas with a lower isolation (i.e., more ponds in the surroundings) bee-richness increased with kettle hole area (Figure 4.2C). This effect putativley depends on the response of large bee species to isolation that responded positively to a high number of analyzing ponds within buffers radii of 1000–2000m that increased the abundance of large-sized wild bees only (Figure 4.7).

Body size can be used as an approximation of foraging distances as it was demonstrated by many studies (e.g. van Nieuwstadt and Ruano Iraheta 1996, Tscharntke and Gathmann 2002, Westphal *et al.* 2006, Greenleaf *et al.* 2007). Based on the classification of Tscharntke and Gathmann (2002), of foraging ranges, we classified our species into: Small = 100–300 m, Medium = 300-600 m and Large = 600–1200 m foraging ranges. Our results suggest that small and medium species might be foraging locally, while large bees are able to fly long distances to forage and therefore a larger number of neighboring kettle holes (less isolation) enhances their abundance.

However, body sizes was not always shown to be a good indicator to infer flight distances. For example, foraging distances of small–medium solitary bees were found to be higher than 1000m from the nest to the foraging sites (Zurbuchen *et al.* 2010). In our study, all solitary wild-bees were small or medium (Figure S4) and were not affected by the degree of isolation (Figure 4.7); while diversity of large bees mainly represented by eusocial bumblebees increased with the number of neighboring kettle holes. In general, the high metabolic requirement of bumblebee, forces them to fly longer distances (Heinrich 1975). However, this can vary among species (Knight *et al.* 2005) and can also be affected by the distribution of resource patches where linear structures increase flight distances (Cresswell *et al.* 1995). In our study area, the high density of kettle holes and croplands of canola, might offer suitable dense patches for bumblebees to forage covering long distances playing an important role and connecting plant communities occurring in these meta-ecosystems, as well as pollinating canola fields.

4.5.4 Habitat heterogeneity and land-use surrounding

The concept of functional heterogeneity assumes that more heterogeneous habitats provide more resources for food, nesting, or dispersal routes fitting the requirement of many species (Fahrig *et al.* 2011). It has been shown that in agroecosystems the amount of high-quality habitats enhance wild-bee diversity (Kennedy *et al.* 2013; Miljanic *et al.* 2018). In contrast, we found a negative effect of habitat heterogeneity on wild-bee diversity, where only the urban cover surrounding the kettle hole (100—300m) showed a significant negative effect. We speculate that the heterogeneity defined in our study as the different biotopes (land use), does not be necessarily represents a higher quality from a wild-bee perspective.

Landscape heterogeneity acts as a filter shaping functional communities of arthropods where simplified landscapes (fewer land cover types) favours large body sizes and generalist feeding species (Gámez-Virués *et al.* 2015). Accordingly, land use intensity affects bee response-traits such as body size, nesting or sociality but with a stronger effect at local scale (Gabriel da Encarnação Coutinho *et al.* 2018). Low wild-bee diversity found in low-quality kettle holes lacking flowers (no nectar availability) (Figure 4.4, Figure 4.5) plus the negative effect of heterogeneity (i.e., mainly the area of sealed urban space) on wild-bee diversity between 100–300m (Figure 4.3A), suggest that habitat heterogeneity has an effect on wild-bee diversity at local scale too. This tendency did not change according functional traits in respect to lecty, sociality and nesting (Table 4.4), suggesting that the strongly negative effect of habitat heterogeneity cannot be mitigated by functional adaptive traits.

4.6 Conclusion

Wetland habitats of kettle holes harbour a relatively high diversity of wild-bee species assuring pollinations services for local croplands and connecting natural plant communities. Habitat heterogeneity had a negative effect on wild-bee diversity at 100–300m buffer, mainly determined by the surrounding sealed urban cover. Local low-quality habitats (no flower resources) were correlated with lower wild-bee diversity in the kettle holes, without an effect of plant cover. Community composition was mainly characterized by medium sized, polylectic, solitary, belowground nesting species, equally affected by habitat heterogeneity at a 100–300m buffer. Interaction between patch area and degree of isolation had an effect on wild-bee diversity, while a decrease of isolation had a positive effect on large-sized bees only.

4.7 Authors contribution

SLG and CL designed the study. SLG, CL, MP collected the data in the field. SLG and CL analyzed the data, SLG, KR, CL, RT and JJ discussed the results; SLG and JJ lead the writing and all authors contributed critically to the drafts and gave final approval for publication.

4.8 Acknowledgements

We would like to thank Carlos Acame, Gabriela Onandia for their valuable help in the field. K. Rupik (University of Bielefeld) and C. Sauer (MNF Berlin) for taxonomical identification. Larissa Schaub for her valuable help with GIS and Kolja Bergholz for sharing his R code to analyze landscape heterogeneity. This work was supported by Deutsche Forschungsgemeinschaft (DFG), in the framework of the BioMove Research training group (DFG-GRK 2118/1).

4.9 Supplementary Material

Table C1: List of bee species and their functional traits found in the kettle holes.
Table C2: Estimate of % plant cover based on Londo scale (Londo 1976).
Table C3: Characteristics of the selected kettle holes and bee diversity characterized by species richness (S) and Shannon Index (H).
Table C4: Details for the model selection based to test the effect of number of kettle holes occurring at different radii (degree of isolation) on bee species richness and abundance.
Table C5: Definitions of bees functional traits taken from Kratschmer *et al.* (2019).
Fig. C1: Color traps used to sample bees in the kettle holes.
Fig. C2: Rarefaction accumulation curve of species based on 9999 permutations.
Fig. C3: Wild-bee diversity according type of cropland.
Fig. C4: Proportion of small, medium and large species according functional traits.
Fig. C5: Relationship between bee species richness according to body sizes with degree of isolation.

CHAPTER 5

General discussion

The main objective of the present thesis was to infer the ecological processes shaping plant communities from biodiversity and key trait patterns under a metacommunity framework. Specifically, I examined the effects of the environment (biotic and abiotic) on community assembly. The first study mainly focused on the response to abiotic factors at a community level. In this study I evaluated whether different type of kettle holes act as environmental factors filtering species assessing the main ecological processes based on the metacommunity paradigms. The second study focused on biotic interactions at a population level, but comparing four species differing in their dispersal and reproductive strategies in relationship with genetic diversity and gene divergence. In the third study, I evaluated wild-bee diversity patterns in relation to environmental factors (i.e., habitat heterogeneity, flower resources) as potential mobile linkers of plant communities.

In Figure 5.1 an overview of the thesis with the main results are reported. The community assembly model was based on Mittelbach and Schemske (2015) which includes speciation-extinction events (upper part Figure 5.1). The first study focused mainly on diversity and trait distribution of plant communities occurring in heterogeneous habitat islands. In this paper, I evaluated whether different type of kettle holes act as environmental factors filtering species shaping the diversity where species sorting (or mass effects) processes (lower part; Figure 5.1: part I) might occur. My results suggest that the types of kettle holes: ephemeral vs. permanent might provide heterogeneous habitats for plant communities. This study, as many in this field, was constrained in time; therefore, I could not evaluate patch dynamics which might be related to interactions among different processes, such as colonization or competition (dashed line arrow on the right side; Figure 5.1). In addition, I evaluated plant functional traits mainly related to dispersal according the types of kettle holes. I found differences in functional traits regarding seed longevity, life span and dispersion (pollen and seeds) between permanent and ephemeral kettle holes. Overall these results suggest that type of kettle holes might act as filters promoting species sorting into heterogeneous habitats, where probably dispersal limitation and niche differentiation processes also occurs.

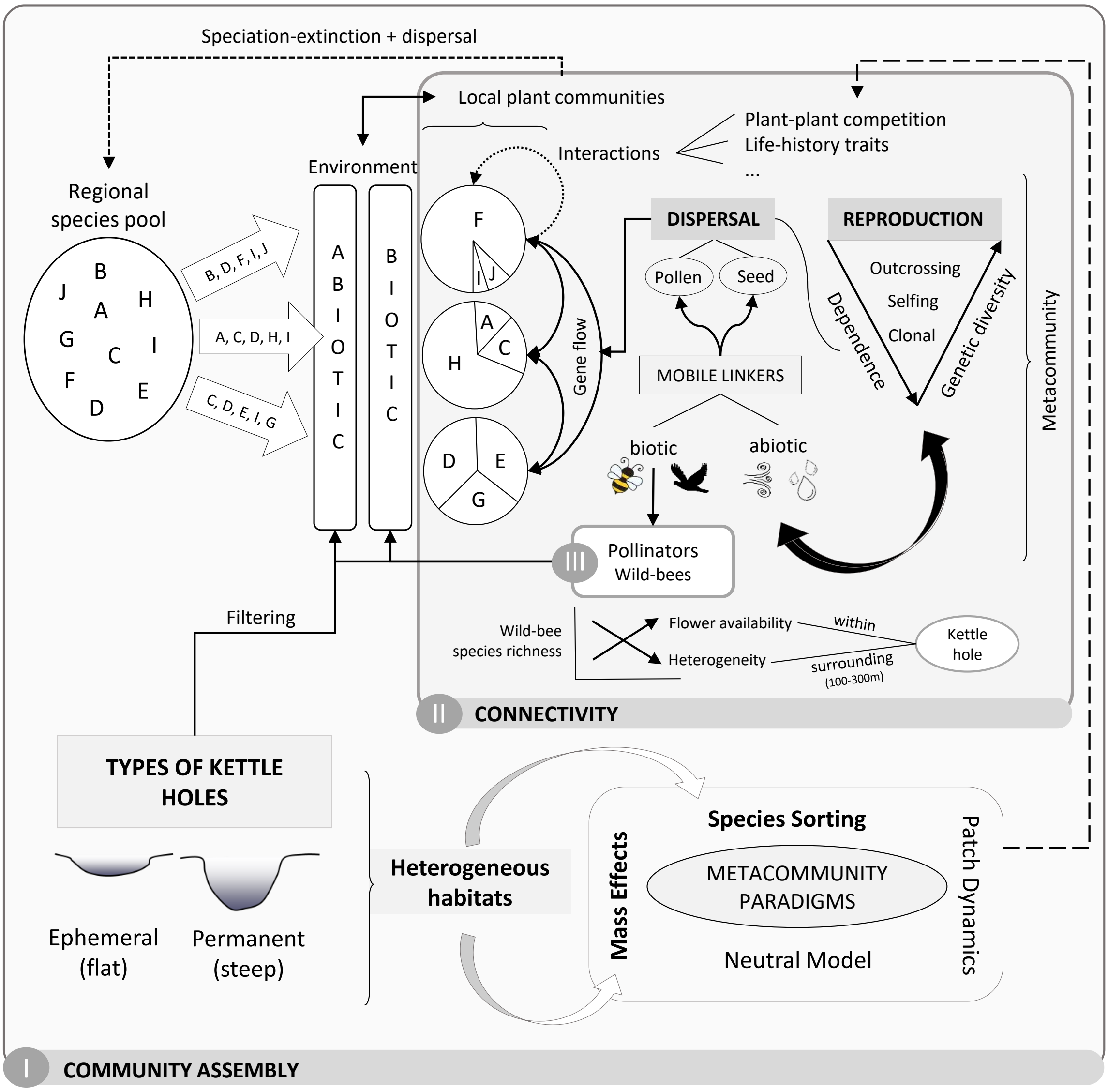


Fig. 5.1. Overview of the thesis structure showing the most relevant results of the corresponding studies. **(I) Community Assembly:** Results suggest that different types of kettle holes act as abiotic filters sorting plant species from the species pool (left lower part). These results combined with comparisons of functional traits, isolation and beta diversity analyses suggest that species sorting and mass effect processes are the main drivers of the community assembly. However, due to time constrain, patch dynamics process could not been evaluated (dashed line right). **(II) Connectivity:** corresponds mainly to specific traits of reproduction and dispersal at a population level. I evaluated the effect of dispersal and reproductive modes on gene flow and genetic diversity. Main results showed the genetic diversity increases in less clonal plants, while the more clonal a species is, the less it depends on dispersal (depicted by the upside down triangle below "reproduction" and its relation to "dispersal"). Gene flow of plants depend on abiotic or biotic "mobile linkers" that transport the seed (i.e. wind) and pollen (i.e., pollinators) to new habitats. **(III) Pollinators wild-bees:** I investigated the influence of environmental factors on wild-bees diversity. Main results showed that wild-bee species richness increased with flower availability within the kettle hole but decrease regarding heterogeneity in the surrounding. The overall plant community assembly model is based on Mittelbach and Schemske (2015).

The second study focused on functional connectivity of four selected species differing in their life-history traits. I compared dispersal and reproductive strategies in relation to genetic diversity and gene divergence. I identified clonal growth as an important trait influencing genetic diversity and gene flow (Figure 5.1, part II: Connectivity). The upside down triangle below “reproduction”, indicates that degree of clonality is increasing from less clonal (outcrossing) to clonal (vegetative), and is positively correlated with dependence of “dispersal” but inversely related to genetic diversity. In other words, the genetic diversity increases in less clonal plants, while the more clonal a species is, the less it depends on dispersal through mobile linkers (i.e., pollinators). Mobile linkers transport pollen and seeds carrying the genes within and between metacommunities. Simultaneously, other mobile linkers (e.g., birds) can affect or be affected by abiotic and biotic environment conditions; and can also influence on the reproduction (or clonal degree), enhancing clonal growth in some cases (i.e., lack of pollinators). This relationship is depicted by the black two way arrow in right side of Figure 5.1, part II.

In the third study, I examined diversity patterns and distribution of functional traits of wild-bees and the effect of habitat heterogeneity (land use), habitat quality (flower resources), patch size and degree of isolation as a response to the environment. Patterns of wild-bee diversity was affected positively by flowers availability but negative correlated to heterogeneity (Figure 5.1: part III). Even though community composition differed regarding sociality, feeding and nesting, no differences in response to environment were detected. However, large sized species mainly characterized by social bumblebees were affected by the degree of isolation (number of neighbor kettle holes in a 1000m buffer). This group of wild-bees might be important pollinators and mobile linkers influencing plant community connectivity across different wetland habitat islands. Therefore, wild-bee biodiversity patterns are important not only to understand their role as potential mobile linkers of plant communities, but also as a functional community at a different level within these complex metaecosystems.

Based on my results, I structured this section in five sections. **First** I discuss specific spatial features of size and proximity and their effects on metacommunities at community and population levels. **Second**, I focus on the response of plant community to abiotic and biotic environmental factors focusing specifically on the role of abiotic filters related to the metacommunity paradigms. In this context, I further discuss the effect of plant-plant interactions and life-history functional traits. **Third**, I concentrate on specific biotic interactions at population level in relationship with the species coexistence theory focusing on the role of clonality and genetic diversity as stabilizing/equalizing mechanisms. **Fourth**, I discuss on the importance of wild-bee diversity and its role as mobile linkers connecting plant communities through pollination services. **Finally** I highlight the importance of integration work as the present one and potential direction for future research that might serve as solid basis for an adequate management and conservation of these intensive agricultural landscapes.

5.1 Size and proximity in metacommunities

In the kettle holes, the island biogeographic theory can be applied due to their condition of "habitat islands". Based on this theory, connectivity is determined by the size and proximity, where species richness is positively related with the area, but negatively related to isolation (MacArthur and Wilson 1967). Therefore, I evaluated the effect of patch area and isolation in my three studies. In the first and third study, I related patch size and isolation directly to species richness, while in the second I compared to inbreeding coefficient (F_{IS}) to patch size and isolation to gene flow (F_{ST}).

In the first study (Chapter 2), I compared patterns of diversity related to patch size (area of the pond) and spatial isolation in permanent vs. ephemeral kettle holes. Both types of ponds presented a positive relationship between species richness and area of the pond, but only ephemeral presented a dependency on the number of neighbor kettle holes (degree of isolation) (Figure 2.2, Table 2.2). The positive island species-area relationship was expected and can be generally explained by the framework of "environmental heterogeneity" where a wider range of habitats is suitable for more different plant species in larger habitats (Stein et al. 2014). Degree of isolation (measured as the number of neighboring kettle holes at different buffers), showed that the more isolated the ephemeral kettle holes are, the less number of species they harbor, this relation was not found for permanent kettle holes. These results suggest that ephemeral kettle holes are more dynamic habitats, likely more vulnerable to agricultural activities and isolation but very important since they might serve as stepping stones enhancing the plant diversity at a regional scale.

In the second study (Chapter 3), I compared inbreeding coefficient (F_{IS}) with patch area. F_{IS} relates observed and expected heterozygosity where negative values indicate excess of heterozygotes and positive values, deficit of heterozygotes. Even though, all species presented a positive relationship between F_{IS} and patch size, patterns differed per species. Non-clonal species *Oenanthe aquatica* presented almost not variation in F_{IS} according to patch size, suggesting that this species is mainly outcrossing. A higher positive relationship between F_{IS} and patch are found in the other three species, which suggests substructure within the populations (Figure 3.3). Positive F_{IS} values in populations of *Typha* and *Phragmites* suggest that larger patches might harbor more (clonal) individuals that would likely mate with closely related individual ramets; while in *Lycopus*, positive F_{IS} might indicate geitenogamous reproduction likely mediated by bees.

Regarding isolation, I evaluated isolation by distance (IBD) relating Fixation index (F_{ST}) as a measurement of gene flow, with the geographic distances between kettle holes. My results showed a difference in wind-pollinated species *Typha* and *Phragmites* vs. insect pollinated species *Lycopus* and *Oenanthe* (Table 3.5), suggesting restricted dispersal in the insect-pollinated species only (Slatkin 1993). However, spatial autocorrelation analyses, that generally should reflect isolation by distance patterns, showed spatial autocorrelation in all species (Figure 3.4). These results support IBD in all species, but at different degree, with a smoother decrease in the outcrossing *Oenanthe* compared with the selfing/clonal species:

Lycopus, *Typha*, and *Phragmites*. Thus, the degree of clonality seems to explain better these results, although there might be some differences regarding dispersal strategies.

It is important to emphasize that the difficulty to conduct direct observation of individual pollen and seed dispersal between populations compel us to make inferences of contemporary gene flow from patterns of genetic diversity. However, these patterns are also influenced by other mechanisms such as genetic drift, metapopulation dynamics and ancestral polymorphisms. This possible mismatch between direct observation of gene flow and population genetic patterns has been identified as "Slatkin's paradox (Slatkin 1987; Marko and Hart 2011). Therefore, inferences of dispersal from contemporary gene flow should be treated carefully.

For the third study (Chapter 4) degree of isolation was measured in the same way as the first study. Results did not show an effect on wild-bee species richness of neither patch size, nor degree of isolation (Figure 4.2). However, a negative relationship between species richness and degree of isolation was found in large body sized bees (Figure 4.7). Diversity of these wild-bees increased with higher number of neighboring kettle holes (less isolation). This group was characterized by mainly social bumblebees, suggesting that these wild-bees might be able to fly longer distances potentially constituting as mobile linkers connecting plant communities occurring in the kettle holes.

5.2 Plant community and populations responses to environmental factors

In the first study, I evaluated the community assembly of plants related to abiotic environmental factors. Abiotic characteristics were based on two key features that divide the kettle holes in two groups: hydroperiod and slope, both related to how often they contain water, dividing the kettle holes in two groups: permanent and ephemeral. As we expected, our results showed that the types of kettle holes (permanent vs. ephemeral), act as strong filters shaping the community. These results suggest that the type of kettle holes provide heterogeneous habitats that might limit the establishment of species. Assuming this, and, under the metacommunity paradigms, Species-Sorting or Mass-Effect processes might occur.

Community composition according type of kettle hole was found to be different than the one expected by chance (Figure 2.3), with an overall high turnover of species suggesting a species sorting process in a regional scale (Table 2.3). Higher turnover in permanent kettle holes and a higher nestedness in ephemeral ones, might indicate that the larger permanent ponds follow the species sorting paradigm harbouring species with a higher replacement than ephemeral ones. The higher nestedness in ephemeral ponds suggests that they are a subset of the species assemblage of the permanent ponds supporting the mass effect process at a local scale (in ephemeral ponds only).

Differences in plant functional traits showed that species are also constrained by dispersal and other life-history traits (such as seed longevity and life span) suggesting niche differentiation between the two types of kettle holes. In ephemeral compared to permanent kettle holes, there was a higher number of short-lived species, longer seed longevity and seed dispersal by animals and humans (hemerochory) (Table 2.4, Figure 2.4). However, disentangling the effect of pure abiotic and biotic condition on communities is a very challenging task. This is because i) multiple processes often operate simultaneously during community assembly (Maire *et al.* 2012; Spasojevic and Suding 2012; Gross *et al.* 2013), and ii) the same trait pattern (e.g. trait convergence), may be generated by different processes such as competition avoidance, dominance, facilitation or environmental filtering (De Bello *et al.* 2012).

Even though differences in diversity and functional traits were found between the types of kettle holes (ephemeral vs. permanent), for the second and third studies, I focused on communities occurring in permanent kettle holes. Permanent kettle holes are longer term habitats whose abiotic characteristics influence species composition and how they respond and adapt to this environment (i.e. high number of long-lived species). The more stable conditions of these type of kettle holes might affect the life cycles of species respect to mating and persistence traits (e.g. seed longevity, clonal growth). But at the same time, due to the island habitat condition of these habitats, dispersal is essential to maintain metacommunities and therefore, dispersal traits play also an important role.

Consequently, on the second study (Chapter 3), I focused on reproduction and dispersal traits of four selected species. I compared whether dispersion or reproduction strategies have a stronger effect on genetic diversity patterns and gene flow. My results suggest that traits mainly related to reproductive strategies: from non-clonal (mainly outcrossing) to clonal (vegetatively), affect genetic diversity and gene flow between metapopulations. Even though we measured genetic diversity and gene flow at population level of four selected species, these traits confer them distinct survival strategies and intraspecific interactions (i.e., competition) with consequences at community level. In the next section I discuss that clonality could act as a stabilizing/equalizing mechanism affecting species coexistence in the community.

5.3 Biotic interactions: stabilizing and equalizing mechanisms for species coexistence

Biotic interactions combined with abiotic factors determine whether species can coexist in the community (HilleRisLambers *et al.* 2012). Based on the Chessonian framework (Chesson 2000), species coexistence is mediated by stabilizing and/or equalizing mechanisms. Stabilizing mechanisms enhance niche differentiation and is based on the abilities of species to respond differently to common limiting factors; while equalizing mechanisms reduce fitness differences. However, niche and fitness are not independent, it is particular difficult to disentangle stabilizing from equalizing mechanisms (Kempel *et al.* 2015; Barabás *et al.* 2018).

5.3.1 Clonality as stabilizing/equalizing mechanism

It has been proposed that clonal growth can enhance species diversity in the community through competition-colonization tradeoffs (Herben *et al.* 1997). Clonal species with ability to grow laterally can occupy larger spaces, be better competitors for nutrients, since they can share them across interconnected ramets, and therefore deal better with disturbances (Klimešová *et al.* 2018). In addition, due to longer persistence on the spot, they possess a higher probability to recover after a damage through resprouting (Ottaviani *et al.* 2017), recolonizing new habitats. However, sometimes competition within individuals of the same species can also occur when distant ramets that became separated come together and compete for resources (Oborny *et al.* 2012).

Clonal growth related to colonization-competition tradeoffs, has indeed been shown in previous experiments in grassland communities (e.g., Wildová *et al.* 2007, Benot *et al.* 2013). According to my results, a higher abundance of the more clonal species (*Typha latifolia* and *Phragmites australis*) than the other two species (*Lycopus europaeus* and *Oenanthe aquatica*) observed within the kettle holes, suggests that the clonal species are better competitors. In addition, a negative relationship between genetic diversity and plant-species richness in the outcrossing species *Oenanthe aquatica* (Figure 3.3, Table 3.4), suggests that this species might suffer from competition (i.e., patches for seedling establishment, pollinators limitation). Therefore, based on my results, clonal growth could promote competition processes and therefore niche differentiation acting as an stabilizing mechanism for species coexistence. Further studies are needed to support this hypothesis.

5.3.2 Genetic diversity as stabilizing/equalizing mechanism

Biodiversity includes phenotypic and genetic variation of individuals of one or more species (Vellend *et al.* 2014). Eco-evolutionary approaches linking species diversity with genetic diversity are relevant to understand biodiversity patterns at different scales (Vellend *et al.* 2014). The study of genetic interactions between species and their environment can allow us to understand complex communities and ecosystems within an evolutionary framework (Whitham *et al.* 2006).

It has been proposed that coexisting genotypes have the potential to show strong fitness differences, and therefore act as an stabilizing mechanism (Chesson 2000). However, other studies have proved that genetic diversity can act as an equalizing mechanism (Nelson *et al.* 2005). Experiments in *Daphnia* populations for instance, have shown that population dynamics provide an equalizing mechanism with strong fitness differences in coexisting genotypes and maintaining genetic diversity (Nelson *et al.* 2005). In our system of wetland plant communities, in order to test whether genetic diversity act either as a stabilizing or equalizing mechanism, analyses of population dynamics and clonal growth will be needed. My results (Chapter 3) showed that genetic diversity was negatively related to degree of clonality, where the mainly outcrossing species (*Oenanthe aquatica*) presented the highest genetic diversity (Figure 3.2, Table 3.3). However, measurements of fitness and population dynamics, are essential to disentangle whether genetic diversity act as stabilizing or equalizing mechanism.

Finally, the influence of intraspecific trait variation on genetic diversity and community assembly should also be considered (Fridley *et al.* 2007; Jung *et al.* 2010; Whitlock *et al.* 2010; De Bello *et al.* 2011; Violle *et al.* 2012; Schöb *et al.* 2015). In addition, intraspecific trait variation is mainly a result of phenotypic plasticity (Vellend *et al.* 2014). Therefore, intraspecific variation may serve as a response to the community instead, where individuals which certain trait genetically determined, would have more (or less) probabilities to join the community (Vellend *et al.* 2014).

In the studied wetland habitats islands, clonal growth seems to have an important effect on genetic diversity and probably on the entire community. Clonal growth can be enhanced by a response to certain environmental stimuli (i.e., absence of pollinators, soil conditions), and therefore contain an element of plasticity which is absent in seed dispersal (Stuefer *et al.* 2001; Klimešová and Klimeš 2008; Zobel *et al.* 2010)

In a plant metacommunity framework, interactions are constrained by plants' lack of mobility (dispersal limitation), with plants occupying new spaces only by dispersal or clonal growth. Patch size and distance to the closest suitable patch are factors expected to restrict clonal mobility and dispersal and therefore influencing the entire community assembly.

5.4 Wild-bee diversity: heterogeneity and mobile linkers

In the ecosystem, complex processes such as predation or herbivory, involve interactions among communities at different levels. Biotic and abiotic factors influence communities of pollinators (e.g. bees), but plant-pollinator mutual interactions can influence each other in a mutual way. For example, plant community composition have an effect on foraging behavior. At the same time, some flowering plants depend on pollinators to carry their pollen for successful reproduction and survival. The later refers to bee species as mobile linkers for plant communities providing pollination services.

In the third study, (Chapter 4), I investigated the relationship between wild-bee diversity to habitat heterogeneity and habitat quality. I hypothesized that habitat heterogeneity affect positively wild-bee diversity based on the assumption that higher habitat diversity enhances wild-bee diversity. Contrary to our expectations, we found a negative correlation between habitat heterogeneity and wild-bee diversity (Figure 4.3), but a positive correlation between wild bee diversity and habitat quality (flower availability) within the ponds (Figure 4.5). Type of land corresponding to urban (houses and streets) had the strongest negative effect on wild-bee diversity between 125-300 m around the kettle holes (Table 4.3).

A positive relationship between flower resources and wild-bee diversity suggest that wild-bees are using the kettle holes as feeding sources and therefore contributing to pollination of plant species occurring there. Taking this into account, it is possible that the plant metacommunities occurring in the kettle holes can sustain the high wild bee diversity that was found, securing natural habitats enhancing genetic diversity of plants and providing with pollination services to croplands as well.

I further evaluated wild-bee community composition using functional traits of feeding, nesting,sociality and body size and assessed whether traits responded similarly to habitat heterogeneity. Differences in community regarding functional traits were found, with most of the species solitary, generalist feeders (polylectic) medium-size and nesting belowground

(Figure 4.6), and their response to habitat heterogeneity (negatively) did not vary significantly (Table 4.4). As it was discussed on section 5.1, an effect of degree of isolation on large body sizes wild-bees was found, which suggests that large sized bumblebees might constitute active mobile linkers connecting plant communities. How bees move, their foraging behavior and flight cover distances can determine genetic flow and structure of plant communities (Waser *et al.* 1996).

Finally, some mobile linkers could also influence communities in an indirect way. As I previously discussed, clonal growth can be an effective response of plants to changing conditions such as absence of pollinators. Therefore, biotic linkers like pollinators can also influence plasticity responses and the assembly of the entire community. At the same time, mobile linkers can serve as gene carriers and therefore have a direct effect on gene diversity of plants.

Overall, my results showed different patterns and interactions at different levels (plants, pollinators) in these island-like habitats. These small water bodies provide long term-stability and microclimatic conditions that facilitate the persistence of population under adverse climatic conditions making them ideal candidates for microrefugia resilient against climate change (Rull 2009; Dobrowski 2011; Keppel and Wardell-Johnson 2015). Trait-based studies can be used to study eco-evolutionary functioning of microrefugia, particularly comparing similar in refugial and non-refugial habitats (e.g., Ottaviani *et al.* 2019). My results provided an overview of functional traits in plant and wild-bee communities, but more detail studies are needed. An integration of communities functional traits with phylogeny would be needed not only to identify the kettle holes as microrefugia (Keppel *et al.* 2018), but also for a better understanding of community assembly (Leibold *et al.* 2010; Gerhold *et al.* 2015).

5.5 Final remarks: integration and further research

A better understanding of the factors affecting assembly of communities and the life-history traits of communities occurring in the kettle holes is crucial for a better conservation and management of these ecosystems. In the context of climate change, is relevant to understand life-history traits related to adaptation to new ecological conditions through plastic responses and dispersal capacity to escape unfavorable conditions to reach more favorable sites (Bornette and Puijalon 2011). In addition, assessing how patterns of dispersal and biodiversity are affected by human activities related to land-use intensity is a the key for good management and conservation of these ecosystems (Zobel 2016). Finally, trait-based studies, phylogenetic and community assembly can help to disentangle eco-evolutionary processes and also identifying these kettle holes as microrefugia.

Understanding the complexity of nature can only be achieved integrating different levels: from genes to ecosystems at different spatiotemporal scales (Tscharntke *et al.* 2012).Teamwork of interdisciplinary research areas from taxonomy to genomics and evolutionary biology (Whitham *et al.* 2006), as well as the use of different techniques. For example, improvement in genetics and sequencing techniques are now being used to infer gene flow at genomic level (e.g., SNPs) or the use of mitochondrial DNA to assess phylogenetics or biodiversity patterns (metabarcoding). Advance in computer power allows us to model more complex ecosystems that in combination with empirical studies, provide us with more accurate predictions.

The present thesis is an example of integrating, both ecological and genetic techniques at different levels: plants and pollinators. It represents a small part of the complex system but an important contribution with insights for future investigations and bases for conservation and management of these metaecosystems.

References

Aavik, T., R. Holderegger, and J. Bolliger. 2014. The structural and functional connectivity of the grassland plant Lychnis flos-cuculi. Heredity 112:471–478

Aavik, T., R. Holderegger, P. J. Edwards, and R. Billeter. 2013. Patterns of contemporary gene flow suggest low functional connectivity of grasslands in a fragmented agricultural landscape. Journal of Applied Ecology 50:395–403.

Agapow, P.-M., and A. Burt. 2001. Indices of multilocus linkage disequilibrium. Molecular Ecology Notes 1:101–102.

Aizen, M. A., G. Gleiser, M. Sabatino, L. J. Gilarranz, J. Bascompte, and M. Verdú. 2016. The phylogenetic structure of plant-pollinator networks increases with habitat size and isolation. Ecology Letters 19:29–36.

Alderton, E., C. D. Sayer, R. Davies, S. J. Lambert, and J. C. Axmacher. 2017. Buried alive: Aquatic plants survive in 'ghost ponds' under agricultural fields. Biological Conservation 212:105–110.

Ally, D., K. Ritland, and S. P. Otto. 2010. Aging in a long-lived clonal tree. PLoS Biology 8:19–20.

Altenfelder, S., J. Kollmann, and H. Albrecht. 2016a. Effects of farming practice on populations of threatened amphibious plant species in temporarily flooded arable fields: Implications for conservation management. Agriculture, Ecosystems and Environment 222:30–37.

Altenfelder, S., U. Raabe, and H. Albrecht. 2014. Effects of water regime and agricultural land use on diversity and species composition of vascular plants inhabiting temporary ponds in northeastern Germany. Tuexenia 34:145–162.

Altenfelder, S., M. Schmitz, P. Poschlod, J. Kollmann, and H. Albrecht. 2016b. Managing plant species diversity under fluctuating wetland conditions: the case of temporarily flooded depressions. Wetlands Ecology and Management 24:597–608.

Auffret, A. G., Y. Rico, J. M. Bullock, D. A. P. Hooftman, A. Traveset, R. J. Pakeman, M. B. Soons, A. Su, H. H. Wagner, and S. A. O. Cousins. 2017. Plant functional connectivity – integrating landscape structure and effective dispersal. Journal of Ecology 105:1648–1656.

Le Bagousse-Pinguet, Y., N. Gross, F. T. Maestre, V. Maire, F. de Bello, C. R. Fonseca, J. Kattge, E. Valencia, J. Leps, and P. Liancourt. 2017. Testing the environmental filtering concept in global drylands. Journal of Ecology:1–12.

Barabás, G., R. D'Andrea, and S. M. Stump. 2018. Chesson's coexistence theory. Ecological Monographs 88:277–303.

Bartelt-Ryser, J., J. Joshi, B. Schmid, H. Brandl, and T. Balser. 2005. Soil feedbacks of plant diversity on soil microbial communities and subsequent plant growth. Perspectives in Plant Ecology, Evolution and Systematics 7:27–49.

Baselga, A., and C. D. L. Orme. 2012. Betapart: An R package for the study of beta diversity. Methods in Ecology and Evolution 3:808–812.

Bazzaz, F. 1996. Plants in changing environments: linking physiological, population and community ecology. Cambridge University Press, Cambridge.

Bekker, R. M., J. P. Bakker, U. Grandin, R. Kalamees, P. Milberg, P. Poschlod, J. H. Willems, R. Kalamees, P. Milberg, P. Poschlod, and J. H. Willems. 1998. Seed size, shape and vertical distribution in the soil: Indicators of seed longevity. Functional Ecology 12:834–842.

De Bello, F., S. Lavorel, C. H. Albert, W. Thuiller, K. Grigulis, J. Dolezal, Š. Janeček, and J. Lepš. 2011. Quantifying the relevance of intraspecific trait variability for functional diversity. Methods in Ecology and Evolution 2:163–174.

De Bello, F., J. N. Price, T. Münkemüller, J. Liir, M. Zobel, W. Thuiller, P. Gerhold, L. Götzenberger, S. Lavergne, J. Lepš, K. Zobel, and M. Pärtel. 2012. Functional species pool framework to test for biotic effects on community assembly. Ecology 93:2263–2273.

Benot, M. L., A. K. Bittebiere, A. Ernoult, B. Clément, and C. Mony. 2013. Fine-scale spatial patterns in grassland communities depend on species clonal dispersal ability and interactions with neighbours. Journal of Ecology 101:626–636.

Bhargava, A., and F. F. Fuentes. 2010. Mutational dynamics of microsatellites. Molecular Biotechnology 44:250–266.

Biggs, J., S. von Fumetti, and M. Kelly-Quinn. 2017. The importance of small waterbodies for biodiversity and ecosystem services: implications for policy makers. Hydrobiologia 793:3–39.

Bommarco, R., J. C. Biesmeijer, B. Meyer, S. G. Potts, J. Pöyry, S. P. M. Roberts, I. Steffan-Dewenter, and E. Ockinger. 2010. Dispersal capacity and diet breadth modify the response of wild bees to habitat loss. Proceedings of the Royal Society B: Biological Sciences 277:2075–2082.

Boonstra, D. K., D. Tsopoglou-Gkina, B. A. Nolet, J. G. B. van Dijk, E. Kleyheeg, T. Y. Woud, and M. B. Soons. 2017. Movement patterns of a keystone waterbird species are highly predictable from landscape configuration. Movement Ecology 5:1–14.

Borcard, D., F. Gillet, and P. Legendre. 2008. Numerical Ecology with R. Page (R. Gentleman, K. Hornik, and G. G. Parmigiani, Eds.) Springer.

Bornette, G., and S. Puijalon. 2011. Response of aquatic plants to abiotic factors: A review. Aquatic Sciences 73:1–14.

Bosch, J., A. M. Martín González, A. Rodrigo, and D. Navarro. 2009. Plant-pollinator networks: Adding the pollinator's perspective. Ecology Letters 12:409–419.

Brinson, M. M. 1993. A hydrogeomorphic classification for wetlands. Wetlands Research Programm Technical Report WRP-DE-4 WRP-DE-4:101.

Brochet, A. L., M. Guillemain, H. Fritz, M. Gauthier-Clerc, and A. J. Green. 2009. The role of migratory ducks in the long-distance dispersal of native plants and the spread of exotic plants in Europe. Ecography 32:919–928.

Brooker, R. W., and T. V. Callaghan. 1998. The balance between positive and negative plant interactions and its relationship to environmental gradients: a model. Oikos 81:196–207.

Brooker, R. W., F. T. Maestre, R. M. Callaway, C. L. Lortie, L. A. Cavieres, G. Kunstler, P. Liancourt, K. Tielbörger, J. m. J. Travis, F. Anthelme, C. Armas, L. Coll, E. Corcket, S. Delzon, E. Forey, Z. Kikvidze, J. Olofsson, F. Pugnaire, C. L. Quiroz, P. Saccone, K. Schiffers, M. Seifan, B. Touzard, and R. Michalet. 2007. Facilitation in plant communities: the past, the present, and the future. Journal of Ecology 96:18–34.

Brose, U. 2001. Relative importance of isolation, area and habitat heterogeneity for vascular plant species richness of temporary wetlands in east-German farmland. Ecography 24:722–730.

Brown, B. L., E. R. Sokol, J. Skelton, and B. Tornwall. 2017. Making sense of metacommunities: dispelling the mythology of a metacommunity typology. Oecologia 183:643–652.

Bullock, J. M., R. E. Kenward, and R. Hails. 2002. Dispersal Ecology. Blackwell Science, Oxford.

Butterfield, B. J., and S. M. Munson. 2016. Temperature is better than precipitation as a predictor of plant community assembly across a dryland region. Journal of Vegetation Science 27:938–947.

De Caceres, M., X. Font, F. Oliva, A. M. De Cáceres, X. Font, F. Oliva, and D. Cáceres. 2008. Assessing species diagnostic value in large data sets: A comparison between phi-coefficient and Ochiai index. Journal of Vegetation Science 19:779–788.

Cadotte, M. W., T. J. Davies, and P. R. Peres-Neto. 2017. Why phylogenies do not always predict ecological differences. Ecological Monographs 87:535–551.

Cahill, J. F., S. W. Kembel, E. G. Lamb, and P. A. Keddy. 2008. Does phylogenetic relatedness influence the strength of competition among vascular plants? Perspectives in Plant Ecology, Evolution and Systematics 10:41–50.

Cain, M. L., B. G. Milligan, and A. E. Strand. 2000. Long-distance seed dispersal in plant populations. American Journal of Botany 87:1217–1227.

Cavender-Bares, J., K. H. Kozak, P. V. A. Fine, and S. W. Kembel. 2009. The merging of community ecology and phylogenetic biology. Ecology letters 12:693–715.

Céréghino, R., J. Biggs, B. Oertli, and S. Declerck. 2008. The ecology of European ponds: Defining the characteristics of a neglected freshwater habitat. Hydrobiologia 597:1–6.

Céréghino, R., B. Oertli, M. Bazzanti, C. Coccia, A. Compin, J. Biggs, N. Bressi, P. Grillas, A. Hull, T. Kalettka, and O. Scher. 2012. Biological traits of European pond macroinvertebrates. Hydrobiologia 689:51–61.

Champion, P. D., D. E. Hofstra, and J. S. Clayton. 2007. Border control for potential aquatic weeds. Stage 3. Weed risk management. Science for Conservation 271:1–41.

Charalambidou, I., L. Santamaría, C. Jansen, and B. A. Nolet. 2005. Digestive plasticity in mallard ducks modulates dispersal probabilities of aquatic plants and crustaceans. Functional Ecology 19:513–519.

Chesson, P. 2000. Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics 31:343–366.

Ciotir, C., M. Dorken, J. Freeland, C. Ciotir, M. Dorken, and J. Freeland. 2013. Preliminary characterization of *Typha latifolia* and *T. angustifolia* from North America and Europe based on novel microsatellite markers identified through next-generation sequencing. Fundamental and Applied Limnology 182:247–252.

Ciotir, C., J. Szabo, and J. Freeland. 2017. Genetic characterization of cattail species and hybrids (*Typha* spp.) in Europe. Aquatic Botany 141:51–59.

Comte, L., J. Murienne, and G. Grenouillet. 2014. Species traits and phylogenetic conservatism of climate-induced range shifts in stream fishes. Nature Communications 5:1–10.

Corbet, S. A. 2000. Compartments in pollination webs. Conservation Biology 14:1229–1231.

Cottenie, K., and L. De Meester. 2004. Metacommunity structure: Synergy of biotic interactions as selective agents and dispersal as fuel. Ecology 85:114–119.

Coutinho, J. G. da E., L. A. Garibaldi, and B. F. Viana. 2018. The influence of local and landscape scale on single response traits in bees: A meta-analysis. Agriculture, Ecosystems & Environment 256:61–73.

Cresswell, J. E., A. P. Bassom, S. A. Bell, S. J. Collins, and T. B. Kelly. 1995. Predicted pollen dispersal by honey-Bees and three species of bumble-bees foraging on oil-seed Rape: A comparison of three models. Functional Ecology 9:829–841.

Cruden, R. W. 1988. Temporal dioecism: systematic breadth, associated traits, and temporal patterns. Botanical Gazette 149:1–15.

Csencsics, D., S. Brodbeck, and R. Holderegger. 2010. Cost-effective, species-specific microsatellite development for the endangered dwarf bulrush (*Typha minima*) using next-generation sequencing technology. Journal of Heredity 101:789–793.

Dafni, A., and D. Firmage. 2000. Pollen viability and longevity: Practical, ecological and evolutionary implications. Plant Systematics and Evolution 222:113–132.

Damschen, E. I., L. A. Brudvig, N. M. Haddad, D. J. Levey, J. L. Orrock, and J. J. Tewksbury. 2008. The movement ecology and dynamics of plant communities in fragmented landscapes. Proceedings of the National Academy of Sciences of the United States of America 105:19078–19083.

Dickerman, J., and R. Wetzel. 1985. Clonal growth in *Typha latifolia*: Population dynamics and demography of the ramets. Journal of Ecology 73:535–552.

Dicks, B. L. V, B. Viana, R. Bommarco, B. Brosi, M. del C. Arizmendi, S. A. Cunningham, L. Galetto, R. Hill, A. V Lopes, C. Pires, H. Taki, and S. G. Potts. 2016. Ten policies for pollinators: What goverments can do to safeguard pollination services. Science 354:975–976.

Dobrowski, S. Z. 2011. A climatic basis for microrefugia: The influence of terrain on climate. Global Change Biology 17:1022–1035.

Dorken, M. E., and C. G. Eckert. 2001. Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodon verticillatus* (Lythraceae). Journal of Ecology 89:339–350.

Dovrat, G., A. Perevolotsky, and G. Ne'eman. 2012. Wild boars as seed dispersal agents of exotic plants from agricultural lands to conservation areas. Journal of Arid Environments 78:49–54.

Earl, D. A., and B. M. VonHoldt. 2012. Structure Harvester: a website and program for visualizing Structure output and implementing the Evanno method. Conservation Genetics Resources 4:359–361.

Eiden, G., M. Kayadjanian, and C. Vidal. 2000. Capturing landscapes structures: Tools. Pages 7–15 From land cover to landscape diversity in the European Union. Brussels, 102 pp.

Ekroos, J., M. Rundlöf, and H. G. Smith. 2013. Trait-dependent responses of flower-visiting insects to distance to semi-natural grasslands and landscape heterogeneity. Landscape Ecology 28:1283–1292.

Ellenberg, H., H. E. Weber, and R. Duell. 1991. Zeigerwerte von Pflanzen in Mitteleuropa = [Indicator values of plants in Central Europe]. Page Scripta geobotanica.

Emerson, B. C., and R. G. Gillespie. 2008. Phylogenetic analysis of community assembly and structure over space and time. Trends in Ecology and Evolution 23:619–630.

Esri. 2011. ArcGIS Desktop: Release 10. USA.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software Stucture: a simulation study. Molecular Ecology 14:2611–2620.

Excoffier, L., and H. E. L. Lischer. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564–567.

Fahrig, L., J. Baudry, L. Brotons, F. G. Burel, T. O. Crist, R. J. Fuller, C. Sirami, G. M. Siriwardena, and J. L. Martin. 2011. Functional landscape heterogeneity and animal biodiversity in agricultural landscapes. Ecology Letters 14:101–112.

Favre-Bac, L., C. Godé, and J.-F. Arnaud. 2014. Characterization of polymorphic microsatellite markers for the fine-leaved water-Dropwort *Oenanthe aquatica* and the Gypsywort *Lycopus europaeus*, two farmland remnant wetland species. Conservation Genetics Resources 6:995–998.

Favre-Bac, L., C. Mony, A. Ernoult, F. Burel, and J. Arnaud. 2016. Ditch network sustains functional connectivity and influences patterns of gene flow in an intensive agricultural landscape. Heredity 116:200–212.

Fénart, S., F. Austerlitz, J. Cuguen, and J. F. Arnaud. 2007. Long distance pollen-mediated gene flow at a landscape level: The weed beet as a case study. Molecular Ecology 16:3801–3813.

Fér, T., and Z. Hroudová. 2009. Genetic diversity and dispersal of *Phragmites australis* in a small river system. Aquatic Botany 90:165–171.

Figuerola, J., and A. J. Green. 2002. Dispersal of aquatic organisms by waterbirds: a review of past research and priorities for future studies. Freshwater Biology 47:483–494.

Figuerola, J., A. J. Green, and L. Santamaría. 2003. Passive internal transport of aquatic organisms by waterfowl in Doñana, south-west Spain. Global Ecology and Biogeography 12:427–436.

Flaherty, K. L., J. S. Rentch, and J. T. Anderson. 2018. Wetland seed dispersal by white-tailed deer in a large freshwater wetland complex. AoB PLANTS 10:1–7.

Fox, J. 2003. Effect Displays in R for Generalised Linear Models. Journal of Statistical Software 8:1–9.

Fraaije, R. G. A. A., C. J. F. Braak, B. Verduyn, J. T. A. A. Verhoeven, B. Soons, C. J. F. ter Braak, B. Verduyn, J. T. A. A. Verhoeven, M. B. Soons, C. J. F. Braak, B. Verduyn, J. T. A. A. Verhoeven, and B. Soons. 2015. Dispersal versus environmental filtering in a dynamic system: Drivers of vegetation patterns and diversity along stream riparian gradients. Journal of Ecology 103:1634–1646.

Fridley, J. D., J. P. Grime, and M. Bilton. 2007. Genetic identity of interspecific neighbours mediates plant responses to competition and environmental variation in a species-rich grassland. Journal of Ecology 95:908–915.

Gabriel da Encarnação Coutinho, J., L. A. Garibaldi, and B. F. Viana. 2018. The influence of local and landscape scale on single response traits in bees: A meta-analysis. Agriculture, Ecosystems & Environment 256:61–73.

Gámez-Virués, S., D. J. Perović, M. M. Gossner, C. Börschig, N. Blüthgen, H. de Jong, N. K. Simons, A.-M. Klein, J. Krauss, G. Maier, C. Scherber, J. Steckel, C. Rothenwöhrer, I. Steffan-Dewenter, C. N. Weiner, W. Weisser, M. Werner, T. Tscharntke, and C. Westphal. 2015. Landscape simplification filters species traits and drives biotic homogenization. Nature Communications 6:8568.

Gerhold, P., J. F. Cahill, M. Winter, I. V. Bartish, and A. Prinzing. 2015. Phylogenetic patterns are not proxies of community assembly mechanisms (they are far better). Functional Ecology 29:600–614.

Gerke, H. H., S. Koszinski, T. Kalettka, and M. Sommer. 2010. Structures and hydrologic function of soil landscapes with kettle holes using an integrated hydropedological approach. Journal of Hydrology 393:123–132.

Gonzalez, A., and E. Chaneton. 2002. Heterotroph species extinction , abundance in an experimentally biomass dynamics fragmented microecosystem. Journal of Animal Ecology 71:594–602.

Gotelli, N. J., and R. K. Colwell. 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. Ecology Letters 4:379–391.

Götzenberger, L., F. de Bello, K. A. Bråthen, J. Davison, A. Dubuis, A. Guisan, J. Lepš, R. Lindborg, M. Moora, M. Pärtel, L. Pellissier, J. Pottier, P. Vittoz, K. Zobel, and M. Zobel. 2012. Ecological assembly rules in plant communities-approaches, patterns and prospects. Biological Reviews 87:111–127.

Gounand, I., E. Harvey, C. J. Little, and F. Altermatt. 2018. Meta-Ecosystems 2.0: Rooting the theory into the field. Trends in Ecology and Evolution 33:36–46.

Graae, B. J., V. Vandvik, W. S. Armbruster, W. L. Eiserhardt, J. Svenning, K. Hylander, J. Ehrlén, J. D. . Speed, K. Klanderud, K. A. Bråthen, A. Milbau, Ø. H. Opedal, I. G. Alsos, R. Ejrnæs, H. H. Bruun, H. J. B. Birks, K. B. Westergaard, H. H. Birks, and J. Lenoir. 2017. Stay or go – how topographic complexity influences alpine plant population and community responses to climate change. Perspectives in Plant Ecology, Evolution and Systematics 30:41–50.

Green, A. J., K. M. Jenkins, D. Bell, P. J. Morris, and R. T. Kingsford. 2008. The potential role of waterbirds in dispersing invertebrates and plants in arid Australia. Freshwater Biology 53:380–392.

Greenleaf, S. S., N. M. Williams, R. Winfree, and C. Kremen. 2007. Bee foraging ranges and their relationship to body size. Oecologia 153:589–596.

Gross, N., L. Börger, S. I. Soriano-Morales, Y. Le Bagousse-Pinguet, J. L. Quero, M. García-Gómez, E. Valencia-Gómez, and F. T. Maestre. 2013. Uncovering multiscale effects of aridity and biotic interactions on the functional structure of Mediterranean shrublands. Journal of Ecology 101:637–649.

Gucker, C. 2008. *Typha latifolia*. In: Fire Effects Information System [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). Available: www.fs.fed.us/database/feis/plants/ [2019, April 8].

Gutzler, C., K. Helming, D. Balla, R. Dannowski, D. Deumlich, M. Glemnitz, A. Knierim, W. Mirschel, C. Nendel, C. Paul, S. Sieber, U. Stachow, A. Starick, R. Wieland, A. Wurbs, and P. Zander. 2015. Agricultural land use changes - A scenario-based sustainability impact assessment for Brandenburg, Germany. Ecological Indicators 48:505–517.

Halkett, F., J. Simon, and F. Balloux. 2005. Tackling the population genetics of clonal and partially clonal organisms. Trends in Ecology & Evolution 20:194–201.

Hallmann, C. A., M. Sorg, E. Jongejans, H. Siepel, N. Hofland, H. Schwan, W. Stenmans, A. Müller, H. Sumser, T. Hörren, D. Goulson, and H. De Kroon. 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLoS ONE 12:1–21.

Harmon-Threatt, A. N., J. H. Burns, L. A. Shemyakina, and T. M. Knight. 2009. Breeding system and pollination ecology of introduced plants compared to their native relatives. American Journal of Botany 96:1544–1550.

Hass, A. L., U. G. Kormann, T. Tscharntke, Y. Clough, A. B. Baillod, C. Sirami, L. Fahrig, J. Martin, J. Baudry, C. Bertrand, J. Bosch, L. Brotons, F. Burel, R. Georges, D. Giralt, M. A. Moarcos-García, A. Ricarte, G. Siriwardena, and P. Batáry. 2018. Landscape configurational heterogeneity by small-scale agriculture, not crop diversity, maintains pollinators and plant reproduction in western Europe. Proceedings of the Royal Society B: Biological Sciences 285:1–10.

Hector, A., J. Joshi, S. Lawler, E. M. Spehn, and A. Wilby. 2001. Conservation implications of the link between biodiversity and ecosystem functioning. Oecologia 129:624–628.

Heinrich, B. 1975. Energetics of Pollination. Annual Missouri Botanical Garden 68:370–378.

Henriques-Silva, R., Z. Lindo, and P. R. Peres-Neto. 2013. A community of metacommunities: Exploring patterns in species distributions across large geographical areas. Ecology 94:627–639.

Herben, T., F. Krahulec, V. Hadincová, S. Pecháčková, T. Herben, F. Krahulec, V. Hadincova, and S. Pechackova. 1997. Fine-scale species interactions of clonal plants in a mountain grassland: A removal experiment. Oikos 78:299.

Hill, M. J., J. Biggs, I. Thornhill, R. A. Briers, D. G. Gledhill, J. C. White, P. J. Wood, and C. Hassall. 2016. Urban ponds as an aquatic biodiversity resource in modified landscapes. Global Change Biology 23:986–999.

Hill, M. J., C. Hassall, B. Oertli, L. Fahrig, B. J. Robson, J. Biggs, M. J. Samways, N. Usio, N. Takamura, J. Krishnaswamy, and P. J. Wood. 2018. New policy directions for global pond conservation. Conservation Letters 11:1–8.

Hill, M. J., J. Heino, I. Thornhill, D. B. Ryves, and P. J. Wood. 2017. Effects of dispersal mode on the environmental and spatial correlates of nestedness and species turnover in pond communities. Oikos 126:1575–1585.

HilleRisLambers, J., P. B. Adler, W. S. Harpole, J. M. Levine, and M. M. Mayfield. 2012. Rethinking community assembly through the lens of Coexistence Theory. Annual Review of Ecology, Evolution, and Systematics 43:227–248.

Hintze, C., F. Heydel, C. Hoppe, S. Cunze, A. König, and O. Tackenberg. 2013. D3: The Dispersal and Diaspore Database - Baseline data and statistics on seed dispersal. Perspectives in Plant Ecology, Evolution and Systematics 15:180–192.

Hoef, J. M. Ver, and P. L. Boveng. 2007. Quasi-poisson vs. Negative Binomial Regression: How should we model overdispersed count data? Ecology 88:2766–2772.

Hroudova, Z., P. Zakravsky, L. Hrouda, and I. Ostry. 1992. *Oenanthe aquatica*: Seed reproduction, population structure, habitat conditions and distribrution in Czechoslovakia. Folia Geobot. Phytotax 27:301–335.

Husband, B., and S. Barret. 2009. A Metapopulation perspective in plant population biology 84:461–469.

IPBES. 2016. The assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination and food production. Page Science. Bonn, Germany.

Jeffries, M. 2008. The spatial and temporal heterogeneity of macrophyte communities in thirty small, temporary ponds over a period of ten years. Ecography 31:765–775.

Jeffries, M. J. 2012. Ponds and the importance of their history: An audit of pond numbers, turnover and the relationship between the origins of ponds and their contemporary plant communities in south-east Northumberland, UK. Hydrobiologia 689:11–21.

Jeltsch, F., D. Bonte, G. Pe'er, B. Reineking, P. Leimgruber, N. Balkenhol, B. Schröder, C. M. Buchmann, T. Mueller, N. Blaum, D. Zurell, K. Böhning-Gaese, T. Wiegand, J. A. Eccard, H. Hofer, J. Reeg, U. Eggers, and S. Bauer. 2013. Integrating movement ecology with biodiversity research - exploring new avenues to address spatiotemporal biodiversity dynamics. Movement Ecology 1:6.

Jiménez-Alfaro, B., L. García-Calvo, P. García, and J. L. Acebes. 2016. Anticipating extinctions of glacial relict populations in mountain refugia. Biological Conservation 201:243–251.

Jung, V., C. Violle, C. Mondy, L. Hoffmann, and S. Muller. 2010. Intraspecific variability and trait-based community assembly. Journal of Ecology 98:1134–1140.

Kalettka, T., G. Berger, P. H, C. Rudat, H. Pfeffer, C. Rudat, P. H, C. Rudat, H. Pfeffer, C. Rudat, P. H, and C. Rudat. 2005. Integrated conservation and management of kettle holes in young moraine agricultural landscapes of northeast Germany. Landscape Research:19–22.

Kalettka, T., and C. Rudat. 2006. Hydrogeomorphic types of glacially created kettle holes in North-East Germany. Limnologica 36:54–64.

Kalettka, T., C. Rudat, and J. Quast. 2001. Potholes in northeast German agro-landscapes: functions, land use impacts, and protection strategies. Pages 291–298 in J. D. Tenhunen, R. Lenz, and R. Hantschel, editors. Ecosystem approaches to landscape management in central Europe, ecological studies. Springer.

Kayler, Z. E., M. Badrian, A. Frackowski, H. Rieckh, K. N. Nitzsche, T. Kalettka, C. Merz, and A. Gessler. 2017. Ephemeral kettle hole water and sediment temporal and spatial dynamics within an agricultural catchment. Ecohydrology 11:1–11.

Kempel, A., M. Razanajatovo, C. Stein, S. B. Unsicker, H. Auge, W. W. Weisser, M. Fischer, and D. Prati. 2015. Herbivore preference drives plant community composition. Ecology 96:2923–2934.

Kennedy, C. M., E. Lonsdorf, M. C. Neel, N. M. Williams, T. H. Ricketts, R. Winfree, R. Bommarco, C. Brittain, A. L. Burley, D. Cariveau, L. G. Carvalheiro, et al., and C. Kremen. 2013. A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. Ecology Letters 16:584–599.

Keppel, G., G. Ottaviani, S. Harrison, G. W. Wardell-Johnson, M. Marcantonio, and L. Mucina. 2018. Towards an eco-evolutionary understanding of endemism hotspots and refugia. Annals of Botany 122:927–934.

Keppel, G., and G. W. Wardell-Johnson. 2015. Refugial capacity defines holdouts, microrefugia and stepping-stones: A response to Hannah et al. Trends in Ecology and Evolution 30:233–234.

Kirk, H., C. Connolly, and J. R. Freeland. 2011. Molecular genetic data reveal hybridization between *Typha angustifolia* and *Typha latifolia* across a broad spatial scale in eastern North America. Aquatic Botany 95:189–193.

Klein, A. M., I. Steffan-Dewenter, and T. Tscharntke. 2003. Fruit set of highland coffee increases with the diversity of pollinating bees. Proceedings of the Royal Society B: Biological Sciences 270:955–961.

Klein, A. M., B. E. Vaissière, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, and T. Tscharntke. 2007. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B: Biological Sciences 274:303-313

Kleyer, M., R. M. Bekker, I. C. Knevel, J. P. Bakker, K. Thompson, M. Sonnenschein, P. Poschlod, J. M. Van Groenendael, L. Klimeš, J. Klimešová, S. Klotz, G. M. et al., and B. Peco. 2008. The LEDA Traitbase: A database of life-history traits of the Northwest European flora. Journal of Ecology 96:1266–1274.

Kleyheeg, E., C. H. A. van Leeuwen, M. A. Morison, B. A. Nolet, and M. B. Soons. 2015. Bird-mediated seed dispersal: Reduced digestive efficiency in active birds modulates the dispersal capacity of plant seeds. Oikos 124:899–907.

Klimešová, J., and F. Bello. 2009. CLO-PLA: the database of clonal and bud bank traits of Central European flora. Journal of Vegetation Science 20:511–516.

Klimešová, J., J. Dolezal, K. Prach, and J. Kosnar. 2012. Clonal growth forms in Arctic plants and their habitat preferences: a study from Petuniabukta, Spitsbergen. Polish Polar Research 33:421–442.

Klimešová, J., T. Herben, and J. Martínková. 2017. Disturbance is an important factor in the evolution and distribution of root-sprouting species. Evolutionary Ecology 31:387–399.

Klimešová, J., and L. Klimeš. 2008. Clonal growth diversity and bud banks of plants in the Czech flora: An evaluation using the CLO-PLA3 database. Preslia 80:255–275.

Klimešová, J., J. Martínková, and T. Herben. 2018. Horizontal growth: An overlooked dimension in plant trait space. Perspectives in Plant Ecology, Evolution and Systematics 32:1–4.

Knight, M. E., A. P. Martin, S. Bishop, J. L. Osborne, R. J. Hale, R. A. Sanderson, and D. Goulson. 2005. An interspecific comparison of foraging range and nest density of four bumblebee (Bombus) species. Molecular Ecology 14:1811–1820.

Kopelman, N. M., J. Mayzel, M. Jakobsson, N. A. Rosenberg, and I. Mayrose. 2015. Clumpak: A program for identifying clustering modes and packaging population structure inferences across K. Molecular Ecology Resources 15:1179–1191.

Kraft, N. J. B., P. B. Adler, O. Godoy, E. C. James, S. Fuller, and J. M. Levine. 2015. Community assembly, coexistence and the environmental filtering metaphor. Functional Ecology 29:592–599.

Kraft, N. J. B., W. K. Cornwell, C. O. Webb, and D. D. Ackerly. 2007. Trait evolution, community assembly, and the phylogenetic structure of ecological communities. The American Naturalist 170:271–283.

Kraft, N. J. B., R. Valencia, and D. D. Ackerly. 2008. Functional traits and niche-based tree community assembl in an amazonian forest. Science 322:580–582.

Kratschmer, S., B. Pachinger, M. Schwantzer, D. Paredes, G. Guzmán, J. A. Goméz, J. A. Entrenas, M. Guernion, F. Burel, A. Nicolai, A. Fertil, D. Popescu, L. Macavei, A. Hoble, C. Bunea, M. Kriechbaum, J. G. Zaller, and S. Winter. 2019. Response of wild bee diversity, abundance, and functional traits to vineyard inter-row management intensity and landscape diversity across Europe. Ecology and Evolution 9:4103–4115.

Kremen, C., N. M. Williams, R. L. Bugg, J. P. Fay, and R. W. Thorp. 2004. The area requirements of an ecosystem service: Crop pollination by native bee communities in California. Ecology Letters 7:1109–1119.

Kühn, I., W. Durka, and S. Klotz. 2004. BiolFlor — a new plant-trait database as a tool for plant invasion ecology. Divers Distrib 10:363–365.

Kurtz, C., and T. Heinken. 2011. Diasporenbankanalyse zum Nachweis gefährdeter Segetalarten auf ehemaligen Ackerstandorten: Keimlingsauflaufverfahren versus Freilandauflaufverfahren. Tuexenia 31:105–126.

Laliberte, E., G. Zemunik, and Benjamin Turner. 2014. Environmental filtering explains variation in plant diversity along resource gradients. Science 345:1602–1605.

Lambert, A. M., and R. A. Casagrande. 2007. Characteristics of a successful estuarine invader: Evidence of self-compatibility in native and non-native lineages of *Phragmites australis*. Marine Ecology Progress Series 337:299–301.

Leibold, M. A., E. P. Economo, and P. Peres-Neto. 2010. Metacommunity phylogenetics: Separating the roles of environmental filters and historical biogeography. Ecology Letters 13:1290–1299.

Leibold, M. A., M. Holyoak, N. Mouquet, P. Amarasekare, J. M. Chase, M. F. Hoopes, R. D. Holt, J. B. Shurin, R. Law, D. Tilman, M. Loreau, and A. Gonzalez. 2004. The metacommunity concept: A framework for multi-scale community ecology. Ecology Letters 7:601–613.

Leimu, R., P. Mutikainen, J. Koricheva, and M. Fischer. 2006. How general are positive relationships between plant population size, fitness and genetic variation? Journal of Ecology 94:942–952.

Li, Y., B. Shipley, J. N. Price, V. D. L. Dantas, R. Tamme, M. Westoby, A. Siefert, B. S. Schamp, M. J. Spasojevic, V. Jung, D. C. Laughlin, S. J. Richardson, Y. Le Bagousse-Pinguet, C. Schöb, et al., and M. A. Batalha. 2018. Habitat filtering determines the functional niche occupancy of plant communities worldwide. Journal of Ecology 106:1001–1009.

Lienert, J. 2004. Habitat fragmentation effects of fitness of plant populations - A review. Journal for Nature Conservation 12:53–72.

Lischeid, G., T. Kalettka, M. Holländer, J. Steidl, C. Merz, R. Dannowski, T. Hohenbrink,

C. Lehr, G. Onandia, F. Reverey, and M. Pätzig. 2017. Natural ponds in an agricultural landscape: External drivers, internal processes, and the role of the terrestrial-aquatic interface. Limnologica 68:5–16.

Lischer, H. E. L., and L. Excoffier. 2012. PGDSpider: An automated data conversion tool for connecting population genetics and genomics programs. Bioinformatics 28:298–299.

Liu, J., M. Wilson, G. Hu, J. Liu, J. Wu, and M. Yu. 2018. How does habitat fragmentation affect the biodiversity and ecosystem functioning relationship? Landscape Ecology 33:341–352.

Logue, J. B., N. Mouquet, H. Peter, and H. Hillebrand. 2011. Empirical approaches to metacommunities: A review and comparison with theory. Trends in Ecology and Evolution 26:482–491.

Londo, G. 1976. The decimal scale for releves of permanent quadrats. Vegetatio 33:61–64.

Loreau, M., N. Mouquet, R. D. Holt, M. Loreau, N. Mouquet, and R. D. Holt. 2003. Meta-ecosystems: A theoretical framework for a spatial ecosystem ecology. Ecology Letters 6:673–679.

Losey, J. E., and M. Vaughan. 2006. The economic value of ecological services provided by insects. BioScience 56:311–323.

Losos, J. B. 2008. Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. Ecology Letters 11:995–1003.

Lowe, W. H., and F. W. Allendorf. 2010. What can genetics tell us about population connectivity? Molecular Ecology 19:3038–3051.

Lozada-Gobilard, S., S. Stang, K. Pirhofer-Walzl, T. Kalettka, T. Heinken, B. Schröder, J. Eccard, and J. Joshi. 2019. Environmental filtering predicts plant-community trait distribution and diversity: Kettle holes as models of meta-community systems. Ecology and Evolution 9:1898–1910.

Ma, M., C. C. Baskin, K. Yu, Z. Ma, and G. Du. 2017. Wetland drying indirectly influences plant community and seed bank diversity through soil pH. Ecological Indicators 80:186–195.

MacArthur, R., and E. Wilson. 1967. The Theory of Island Biogeography. Princeton Univ. Press, Princeton, New Jersey.

Mahaut, L., G. Fried, and S. Gaba. 2018. Patch dynamics and temporal dispersal partly shape annual plant communities in ephemeral habitat patches. Oikos 127:147–159.

Maire, V., N. Gross, L. Börger, R. Proulx, C. Wirth, L. da S. Pontes, J. F. Soussana, and F. Louault. 2012. Habitat filtering and niche differentiation jointly explain species relative abundance within grassland communities along fertility and disturbance gradients. New Phytologist 196:497–509.

Manel, S., and R. Holderegger. 2013. Ten years of landscape genetics. Trends in Ecology and Evolution 28:614–621.

Marko, P. B., and M. W. Hart. 2011. The complex analytical landscape of gene flow inference. Trends in Ecology and Evolution 26:448–456.

Marques, A., I. S. Martins, T. Kastner, C. Plutzar, M. C. Theurl, N. Eisenmenger, M. A. J. Huijbregts, R. Wood, K. Stadler, M. Bruckner, J. Canelas, J. P. Hilbers, A. Tukker, K. Erb, and H. M. Pereira. 2019. Increasing impacts of land use on biodiversity and carbon sequestration driven by population and economic growth. Nature Ecology & Evolution 3:628–637.

Maxwell, S. L., R. A. Fuller, T. M. Brooks, and J. E. M. Watson. 2016. Biodiversity: The ravages of guns, nets and bulldozers. Nature 536:143–145.

Mayfield, M. M., and J. M. Levine. 2010. Opposing effects of competitive exclusion on the phylogenetic structure of communities. Ecology Letters 13:1085–1093.

McGarigal, K., S. Cushman, and C. Regan. 2005. Quantifying terrestrial habitat loss and fragmentation: A protocol. Landscape, 140 pp.

McGill, B. J., B. J. Enquist, E. Weiher, and M. Westoby. 2006. Rebuilding community ecology from functional traits. Trends in Ecology and Evolution 21:178–185.

De Meester, L., S. Declerck, R. Stoks, G. Louette, F. van de Meutter, T. De Bie, E. Michels, and

L. Brendonck. 2005. Ponds and pools as model systems in conservation biology , ecology and evolutionary biology. Aquatic Conservation: Marine and Freshwater Ecosystems 15:715–725.

Meirmans, P. G., S. Liu, and P. H. Van Tienderen. 2018. The analysis of polyploid genetic data. Journal of Heredity 109:283–296.

Mittelbach, G. G., and D. W. Schemske. 2015. Ecological and evolutionary perspectives on community assembly. Trends in Ecology and Evolution 30:241–247.

Moon, H. K., and S. P. Hong. 2006. Nutlet morphology and anatomy of the genus Lycopus (Lamiaceae: Mentheae). Journal of Plant Research 119:633–644.

Müller-Nilsson, A. 2018. Der Einfluss landschaflicher Vielfalt, standörtlicher Bodeneigenschaften und landwirtschaflichen Managements auf de Diversität und Artenzusammensetzung der Segetalflora im Quillowgebiet. Berlin Technische Universität.

Münkemüller, T., L. Gallien, S. Lavergne, J. Renaud, C. Roquet, S. Abdulhak, S. Dullinger, L. Garraud, A. Guisan, J. Lenoir, J. C. Svenning, J. Van Es, P. Vittoz, W. Willner, T. Wohlgemuth, N. E. Zimmermann, and W. Thuiller. 2014. Scale decisions can reverse conclusions on community assembly processes. Global Ecology and Biogeography 23:620–632.

Nathan, R., W. M. Getz, E. Revilla, M. Holyoak, R. Kadmon, D. Saltz, and P. E. Smouse. 2008. A movement ecology paradigm for unifying organismal movement research. Proceedings of the National Academy of Sciences 105:19052–19059.

Nelson, W. a, E. McCauley, and F. J. Wrona. 2005. Stage-structured cycles promote genetic diversity in a predator-prey system of Daphnia and algae. Nature 433:413–417.

Ngo, H. T., J. Gibbs, T. Griswold, and L. Packer. 2013. Evaluating bee (Hymenoptera: Apoidea) diversity using Malaise traps in coffee landscapes of Costa Rica. Canadian Entomologist 145:435–453.

van Nieuwstadt, M. G. L., and C. E. Ruano Iraheta. 1996. Relation between size and foraging range in stingless bees (Apidae, Meliponinae). Apidologie 27:219–228.

Ning, Y., G.-J. Wu, H. Ma, J.-L. Guo, M.-Y. Zhang, W. Li, Y.-F. Wang, and S.-L. Duoerji. 2018. Contrasting fine-scale genetic structure of two sympatric clonal plants in an alpine swampy meadow featured by tussocks. PLOS ONE 13:1–16.

Nitzsche, K. N., T. Kalettka, K. Premke, G. Lischeid, A. Gessler, and Z. E. Kayler. 2017. Land-use and hydroperiod affect kettle hole sediment carbon and nitrogen biogeochemistry. Science of The Total Environment 574:46–56.

Oborny, B., C. Mony, and T. Herben. 2012. From virtual plants to real communities: A review of modelling clonal growth. Ecological Modelling 234:3–19.

Ochiai, A. 1957. Zoogeographical Studies on the Soleoid Fishes Found in Janpan and its Neighbouring Regions II. Bulletin Of The Japanese Society Of Scientific Fisheries 33:33–40.

Oertli, B., D. A. Joye, E. Castella, R. Juge, D. Cambin, and J. B. Lachavanne. 2002. Does size matter? The relationship between pond area and biodiversity. Biological Conservation 104:59–70.

Ollerton, J., R. Winfree, and S. Tarrant. 2011. How many flowering plants are pollinated by animals? Oikos 120:321–326.

Onandia, G., G. Lischeid, T. Kalettka, A. Kleeberg, M. Omari, K. Premke, and G. B. Arhonditsis. 2018. Biogeochemistry of natural ponds in agricultural landscape: Lessons learned from modeling a kettle hole in Northeast Germany. Science of the Total Environment 634:1615–1630.

Ottaviani, G., G. Keppel, M. Marcantonio, L. Mucina, and G. Wardell-Johnson. 2019. Woody species in resource-rich microrefugia of granite outcrops display unique functional signatures. Austral Ecology 44:575–580.

Ottaviani, G., J. Klimešová, J. G. Pausas, T. Herben, and J. Martínková. 2017. On Plant Modularity Traits: Functions and Challenges. Trends in Plant Science 22:648–651.

Ottaviani, G., M. Marcantonio, and L. Mucina. 2016. Soil depth shapes plant functional diversity in granite outcrops vegetation of Southwestern Australia. Plant Ecology and Diversity 9:263–276.

Ouborg, N. J., Y. Piquot, and J. M. Van Groenendael. 1999. Population genetics, molecular markers and the study of dispersal in plants. Journal of Ecology 87:551–568.

Packer, J. G., L. A. Meyerson, H. Skálová, P. Pyšek, and C. Kueffer. 2017. Biological Flora of the British Isles: *Phragmites australis*. Journal of Ecology 105:1123–1162.

Patzig, M., T. Kalettka, M. Glemnitz, and G. Berger. 2012. What governs macrophyte species richness in kettle hole types? A case study from Northeast Germany. Limnologica 42:340–354.

Peakall, R., and P. E. Smouse. 2012. GenAlex 6.5: Genetic analysis in Excel. Population genetic software for teaching and research- and update. Bioinformatics 28:2537-2539.

Platen, R., T. Kalettka, and C. Ulrichs. 2016. Kettle Holes in the agrarian landscape: Isolated and ecological unique habitats for carabid beetles (Col.: Carabidae) and spiders (Arach.: Araneae). Journal of Landscape Ecology 9:29–60.

Porras-Hurtado, L., Y. Ruiz, C. Santos, C. Phillips, A. Carracedo, M. V. Lareu, Q. Zhang, L. Porras-Hurtado, Y. Ruiz, C. Santos, C. Phillips, A. Carracedo, M. V. Lareu, and Q. Zhang. 2013. An overview of Structure: applications, parameter settings, and supporting software. Frontiers in Genetics 4:1–13.

Poschlod, P., and S. Rosbakh. 2018. Mudflat species: Threatened or hidden? An extensive seed bank survey of 108 fish ponds in Southern Germany. Biological Conservation 225:154–163.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Putman, A. I., and I. Carbone. 2014. Challenges in analysis and interpretation of microsatellite data for population genetic studies. Ecology and Evolution 4:4399–4428.

R. 2018. R core team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www. R-project. org:ISBN 3-900051-07-0, URL http://www.R-project.org/.

Ramankutty, N., A. T. Evan, C. Monfreda, and J. A. Foley. 2008. Farming the planet: 1. Geographic distribution of global agricultural lands in the year 2000. Global Biogeochemical Cycles 22:1–19.

Regal, P. J. 1982. Pollination by Wind and Animals: Ecology of Geographic Patterns. Annual Review of Ecology and Systematics 13:497–524.

Ristow, M., A. Herrmann, H. Illig, H.-C. Klage, G. Klemm, V. Kummer, B. Machatzi, S. Rätzel, R. Schwarz, F. Zimmermann, T. Rote, L. Der, G. Brandenburgs, M. Zahn, H. Nägeli, G. Schneid, H. F. W. Schultz, S. Bip, L. L. Desf, N. All, and A. Waldst. 2006. Liste und Rote Liste der etablierten Gefäßpflanzen Brandenburgs. Naturschutz und Landschaftspflege in Brandenburg 15:163.

Roberts, D. G., C. N. Forrest, A. J. Denham, and D. J. Ayre. 2017. Clonality disguises the vulnerability of a threatened arid zone Acacia. Ecology and Evolution 7:9451–9460.

Rothmaler, W. 2011. Exkursionsflora von Deutschland. Gefäßpflanzen: Grundband. Page (E. J. Jäger, Ed.). 20. Spektrum Akademischer Verlag, Heidelberg.

Roulston, T. H., and K. Goodell. 2011. The role of rsources and risks in regulating wild bee populations. Annual Review of Entomology 56:293–312.

Ruhí, A., T. Datry, and J. L. Sabo. 2017. Interpreting beta-diversity components over time to conserve metacommunities in highly dynamic ecosystems. Conservation Biology 31:1459–1468.

Rull, V. 2009. Microrefugia. Journal of Biogeography 36:481–484.

Saltonstall, K. 2003. Microsatellite variation within and among North American lineages of *Phragmites australis*. Molecular Ecology 12:1689–1702.

Saltonstall, K., K. Glennon, A. Burnett, R. B. Hunter, and K. L. Hunter. 2007. Comparison of Morphological Variation Indicative of Ploidy Level in *Phragmites australis* (Poaceae) From Eastern North America. Rhodora 109:415–429.

Sánchez-Bayo, F., and K. A. G. Wyckhuys. 2019. Worldwide decline of the entomofauna: A review of its drivers. Biological Conservation 232:8–27.

Schermer, É., M. Bel-Venner, D. Fouchet, A. Siberchicot, V. Boulanger, T. Caignard, M. Thibaudon, G. Oliver, M. Nicolas, J. Gaillard, S. Delzon, and S. Venner. 2019. Pollen limitation as a main driver of fruiting dynamics in oak populations. Ecology Letters 22:98–107.

Schirmel, J., J. Thiele, M. H. Entling, and S. Buchholz. 2016. Trait composition and functional diversity of spiders and carabids in linear landscape elements. Agriculture, Ecosystems and Environment 235:318–328.

Schmid, B., J. Joshi, and F. Schläpfer. 2002. Empirical evidence for biodiversity-ecosystem functioning relationships. Pages 120–150 in A. P. Kinzig, S. W. Pacala, and D. Tilman, editors. Functional consequences of biodiversity: empirical progress and theoretical extensions. Princeton University Press.

Schöb, C., S. Kerle, A. J. Karley, L. Morcillo, R. J. Pakeman, A. C. Newton, and R. W. Brooker. 2015. Intraspecific genetic diversity and composition modify species-level diversity productivity relationships. New Phytologist 205:720–730.

Schöb, C., R. Michalet, L. A. Cavieres, F. I. Pugnaire, R. W. Brooker, B. J. Butterfield, B. J. Cook, Z. Kikvidze, C. et al. and R. M. Callaway. 2014. A global analysis of bidirectional interactions in alpine plant communities shows facilitators experiencing strong reciprocal fitness costs. New Phytologist 202:95–105.

Schwarzer, C. 2018. Climate change, adaptive divergence and their effects on species interactions in European bog-plant communities. University of Potsdam.

Selkoe, K. A., and R. J. Toonen. 2006. Microsatellites for ecologists: A practical guide to using and evaluating microsatellite markers. Ecology Letters 9:615–629.

Settele, J., M. Woyciechowski, H. Szentgyörgyi, W. Celary, M. Wantuch, D. Moroń, and C. Westphal. 2008. Diversity of wild bees in wet meadows: Implications for conservation. Wetlands 28:975–983.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. The American Journal of Tropical Medicine and Hygiene 236:787–792.

Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47:264–279.

Soltis, P. S., and D. E. Soltis. 2000. The role of genetic and genomic attributes in the success of polyploids. Proc. Natl. Acad. Sci. U.S.A. 97:7051–7057.

Soons, M. B., A. L. Brochet, E. Kleyheeg, and A. J. Green. 2016. Seed dispersal by dabbling ducks: an overlooked dispersal pathway for a broad spectrum of plant species. Journal of Ecology 104:443–455.

Spasojevic, M. J., and K. N. Suding. 2012. Inferring community assembly mechanisms from functional diversity patterns: The importance of multiple assembly processes. Journal of Ecology 100:652–661.

Staddon, P., Z. Lindo, P. D. Crittenden, F. Gilbert, and A. Gonzalez. 2010. Connectivity, non-random extinction and ecosystem function in experimental metacommunities. Ecology Letters 13:543–552.

Steffan-Dewenter, I., U. Müzenberg, C. Bürger, C. Thies, and T. Tscharntke. 2002. Scale-dependent effects of landscape context on three pollinator guilds. Ecology 83:1421–1432.

Steffan-Dewenter, I., and T. Tscharntke. 1999. Effects of habitat isolation on pollinator communities and seed set. Oecologia 121:432–440.

Stein, A., K. Gerstner, and H. Kreft. 2014. Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales. Ecology Letters 17:866–880.

Stueffer, J. F., H. de Kroon, and H. J. During. 1996. Exploitation of environmental heterogeneity by spatial division of labour in a clonal plant. Functional Ecology 10:328–334.

Taylor, P. D., L. Fahrig, K. Henein, and G. Merriam. 1993. Connectivity is a vital element of landscape structure. Oikos 68:571.

Tiner, R. W. 2003. Geographically isolated wetlands of the United States. Wetlands 23:494–516.

Tischendorf, L., and L. Fahrig. 2000. On the usage and measurement of landscape connectivity. Oikos 90:7–19.

Tscharntke, T., and R. Brandl. 2004. Plant-insect interaction in fragmented landscapes. Annual Review of Entomology 49:405–430.

Tscharntke, T., and A. Gathmann. 2002. Foraging ranges of solitary bees. Journal of Animal Ecology 71:757–764.

Tscharntke, T., J. M. Tylianakis, T. A. Rand, R. K. Didham, L. Fahrig, P. Batáry, J. Bengtsson, Y. Clough, T. O. Crist, C. F. Dormann, R. M. Ewers, J. Fründ, R. D. Holt, et al. and C. Westphal. 2012. Landscape moderation of biodiversity patterns and processes - eight hypotheses. Biological Reviews 87:661–685.

Tsyusko-Omeltchenko, O. V., N. A. Schable, M. H. Smith, and T. C. Glenn. 2003. Microsatellite loci isolated from narrow-leaved cattail *Typha angustifolia*. Molecular Ecology Notes 3:535–538.

Tsyusko, O. V., M. H. Smith, R. R. Sharitz, and T. C. Glenn. 2005. Genetic and clonal diversity of two cattail species, *Typha latifolia* and *T. angustifolia* (Typhaceae), from Ukraine. American Journal of Botany 92:1161–1169.

Ungaro, F., I. Zasada, and A. Piorr. 2014. Mapping landscape services, spatial synergies and trade-offs. A case study using variogram models and geostatistical simulations in an agrarian landscape in North-East Germany. Ecological Indicators 46:367–378.

Vellend, M., G. Lajoie, A. Bourret, C. Múrria, S. W. Kembel, and D. Garant. 2014. Drawing ecological inferences from coincident patterns of population- and community-level biodiversity. Molecular Ecology 23:2890–2901.

Venter, O., E. W. Sanderson, A. Magrach, J. R. Allan, J. Beher, K. R. Jones, H. P. Possingham, W. F. Laurance, P. Wood, B. M. Fekete, M. A. Levy, and J. E. M. Watson. 2016. Sixteen years of change in the global terrestrial human footprint and implications for biodiversity conservation. Nature Communications 7:1–11.

Viana, D. S., J. Figuerola, K. Schwenk, M. Manca, A. Hobæk, M. Mjelde, C. D. Preston, R. J. Gornall, J. M. Croft, R. A. King, A. J. Green, and L. Santamaría. 2016. Assembly mechanisms determining high species turnover in aquatic communities over regional and continental scales. Ecography 39:281–288.

Vickruck, J. L., L. R. Best, M. P. Gavin, J. H. Devries, and P. Galpern. 2019. Pothole wetlands provide reservoir habitat for native bees in prairie croplands. Biological Conservation 232:43–50.

Violle, C., B. J. Enquist, B. J. McGill, L. Jiang, C. H. Albert, C. Hulshof, V. Jung, and J. Messier. 2012. The return of the variance: intraspecific variability in community ecology. Trends in Ecology & Evolution 27:244–252.

Wan, H., S. Cushman, and J. Ganey. 2018. Habitat fragmentation reduces genetic diversity and connectivity of the Mexican spotted owl: A simulation study using empirical resistance models. Genes 9:403.

Waser, N., L. Chittka, M. V. Price, N. M. Williams, and J. Ollerton. 1996. Generalization in pollination systems, and why it matters. Ecology 77:1043–1060.

Watkinson, A. R., and R. P. Freckleton. 2002. Large-scale spatial dynamics of plants: Metapopulations, regional ensembles and patchy populations. Journal of Ecology 90:419–434.

Webb, C. O., D. D. Ackerly, M. A. McPeek, and M. J. Donoghue. 2002. Phylogenies and Community Ecology. Annual Review of Ecology and Systematics 33:475–505.

Weigelt, P., and H. Kreft. 2013. Quantifying island isolation - insights from global patterns of insular plant species richness. Ecography 36:417–429.

Weiher, E., and P. A. Keddy. 1995. Assembly rules, null models, and trait dispersion: new questions from old patterns. Oikos 74:159–164.

Westberg, E., H. H. Poppendieck, and J. W. Kadereit. 2010. Ecological differentiation and reproductive isolation of two closely related sympatric species of *Oenanthe* (Apiaceae). Biological Journal of the Linnean Society 101:526–535.

Westphal, C., R. Bommarco, G. Carré, E. Lamborn, N. Morison, T. Petanidou, S. G. Potts, S. P. M. Roberts, H. Szentgyörgyi, T. Tscheulin, B. E. Vaissière, M. Woyciechowski, J. C. Biesmeijer, W. E. Kunin, J. Settele, and I. Steffan-Dewenter. 2008. Measuring bee diversity in different european habitats and biogeographical regions. Ecological Monographs 78:653–671.

Westphal, C., I. Steffan-Dewenter, and T. Tscharntke. 2003. Mass flowering crops enhance pollinator densities at a landscape scale. Ecology Letters 6:961–965.

Westphal, C., I. Steffan-Dewenter, and T. Tscharntke. 2006. Bumblebees experience landscapes at different spatial scales: Possible implications for coexistence. Oecologia 149:289–300.

Whitehead, D. R. 1969. Wind pollination in the angiosperms: evolutionary and environmental considerations. Evolution 23:28.

Whitham, T. G., J. K. Bailey, J. A. Schweitzer, S. M. Shuster, R. K. Bangert, C. J. LeRoy, E. V Lonsdorf, G. J. Allan, S. P. DiFazio, B. M. Potts, D. G. Fischer, C. A. Gehring, R. L. Lindroth, J. C. Marks, S. C. Hart, G. M. Wimp, and S. C. Wooley. 2006. A framework for community and ecosystem genetics: from genes to ecosystems. Nat Rev Genet 7:510–523.

Whitlock, R. A. J., J. P. Grime, and T. Burke. 2010. Genetic variation in plant morphology contributes to the species-level structure of grassland communities. Ecology 91:1344–1354.

Wildová, R., J. Wild, and T. Herben. 2007. Fine-scale dynamics of rhizomes in a grassland community. Ecography 30:264–276.

Willis, C. G., B. Ruhfel, R. B. Primack, A. J. Miller-Rushing, and C. C. Davis. 2008. Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change. PNAS 105:17029–17033.

Wilson, D. 1992. Complex interactions in metacommunities , with implications for biodiversity and higher levels of selection. Ecology 73:1984–2000.

Wittmann, M. E., M. A. Barnes, C. L. Jerde, L. A. Jones, and D. M. Lodge. 2016. Confronting species distribution model predictions with species functional traits. Ecology and Evolution 6:873–879.

Woodcock, B. A., M. P. D. Garratt, G. D. Powney, R. F. Shaw, J. L. Osborne, J. Soroka, S. A. M. Lindström, D. Stanley, P. Ouvrard, M. E. Edwards, F. Jauker, M. E. McCracken, et al., and R. F. Pywell. 2019. Meta-analysis reveals that pollinator functional diversity and abundance enhance crop pollination and yield. Nature Communications 10:1481.

Yang, D., Y. Song, J. Ma, P. Li, H. Zhang, M. R. S. Price, C. Li, and Z. Jiang. 2016. Stepping-stones and dispersal flow: establishment of a meta-population of Milu (Elaphurus davidianus) through natural re-wilding. Scientific Reports 6:1–10.

Zhou, B., D. Yu, Z. Ding, and X. Xu. 2016. Comparison of genetic diversity in four *Typha* species (Poales, Typhaceae) from China. Hydrobiologia 770:117–128.

Zobel, M. 2016. The species pool concept as a framework for studying patterns of plant diversity. Journal of Vegetation Science 27:8–18.

Zobel, M., M. Moora, and T. Herben. 2010. Clonal mobility and its implications for spatio-temporal patterns of plant communities: What do we need to know next? Oikos 119:802–806.

Zurbuchen, A., L. Landert, J. Klaiber, A. Müller, S. Hein, and S. Dorn. 2010. Maximum foraging ranges in solitary bees: only few individuals have the capability to cover long foraging distances. Biological Conservation 143:669–676.

APPENDIX A

Supplementary Material to Chapter 2

List of tables

List of figures

Table A1: Characteristics of the kettle holes including geographical location, type, area, number of of neighboring kettle holes within a radius of 500 m (using ArcGIS 10; ESRI 2011), surrounding crop, presence of trees, total number of plant species.

ID	Type	Area [m^2]	# Neighbors	Longitude_X	Latitude_Y	Crop	Trees	# species
S010	Steep permanent	1620	3	3402490.822	5907660.721	cereal	Yes	72
S011	Steep permanent	860	8	3419582.472	5912196.614	rape	Yes	43
S012	Flat ephemeral	5560	2	3415332.817	5913951.378	cereal	No	22
S013	Steep permanent	1960	12	3406282.85	5914152.231	others	Yes	56
S014	Steep permanent	700	13	3406584.915	5913623.503	others	Yes	42
S015	Flat ephemeral	2240	22	3408589.986	5911508.778	cereal	No	51
S016	Steep permanent	1370	20	3408804.441	5911785.319	cereal	Yes	48
S017a	Steep permanent	5050	18	3408898.742	5911857.642	cereal	No	51
S017b	Flat ephemeral	1370	15	3408939.565	5911942.263	cereal	No	36
S018	Flat ephemeral	2150	5	3417668.16	5909431.025	cereal	No	28
S019	Steep permanent	2720	8	3400763.977	5908121.123	rape	No	51
S020	Flat ephemeral	870	16	3401822.475	5908467.474	maize	No	35
S021	Flat ephemeral	800	14	3401887.519	5908559.639	cereal	No	41
S022	Steep permanent	650	6	3415858.193	5907966.028	cereal	No	45
S023	Steep permanent	2380	11	3414978.263	5908333.186	cereal	No	69
S024	Steep permanent	1510	5	3414855.188	5909499.901	cereal	Yes	47
S025	Steep permanent	290	4	3414854.506	5909574.017	cereal	No	34
S026	Steep permanent	1840	8	3419206.818	5915652.218	cereal	No	36
S027	Steep permanent	5560	7	3419288.853	5916308.599	cereal	Yes	60
S028	Steep permanent	4740	3	3422334.224	5915910.9	cereal	Yes	35
S029	Steep permanent	540	6	3412607.454	5909410.554	cereal	No	44
S030	Steep permanent	4860	4	3415291.084	5913345.132	cereal	Yes	54
S031	Flat ephemeral	1070	4	3412082.507	5908609.363	cereal	No	16
S032	Steep permanent	470	2	3411578.082	5911426.03	maize	No	23
S033	Steep permanent	700	10	3416594.18	5914275.245	maize	Yes	40
S034	Steep permanent	860	1	3411453.948	5914155.465	cereal	No	53
S035	Steep permanent	590	14	3411166.77	5915019.121	cereal	No	40
S036	Flat ephemeral	4250	9	3410453.343	5918540.624	cereal	No	53
S037	Steep permanent	3910	10	3411674.342	5916589.685	rape	Yes	56
S038	Flat ephemeral	2930	18	3407666.493	5917754.869	rape	No	63
S039	Steep permanent	760	18	3407658.364	5917669.167	rape	Yes	37
S040	Flat ephemeral	300	10	3407224.132	5916554.938	maize	No	29
S041	Steep permanent	2490	8	3408551.963	5916811.128	others	No	63
S042	Flat ephemeral	560	8	3409296.994	5914596.012	cereal	No	32
S043	Steep permanent	680	3	3404454.433	5912173.028	maize	Yes	58
S044	Flat ephemeral	670	0	3422094.256	5912619.964	maize	No	15
S045	Steep permanent	450	9	3402797.079	5912318.703	cereal	Yes	32
S046	Flat ephemeral	2300	12	3403107.908	5911923.833	maize	No	21
S047	Flat ephemeral	240	7	3403368.429	5910698.084	cereal	No	18
S048	Steep permanent	630	3	3403681.123	5909313.416	others	No	39
S049	Flat ephemeral	1510	13	3406103.879	5905435.088	cereal	No	36
S050	Flat ephemeral	390	11	3412616.188	5917021.627	cereal	No	24
S051	Steep permanent	5740	15	3411401.437	5917211.765	cereal	Yes	90
S052	Flat ephemeral	1920	17	3407323.457	5915780.126	maize	No	47
S053	Flat ephemeral	440	11	3406574.478	5914209.382	cereal	No	37
S054	Steep permanent	8470	15	3413818.749	5915121.68	rape	No	63

Table A2: Characteristics of the plant species including colonization and dispersal traits. The seed longevity index ranged from short-lived seeds = 0 to long lived = 1 (Bekker et al. 1998). These data as well as data on species longevity (short=annual/biennial; long=perennial) were taken from the LEDA database (www.uni-oldenburg.de/en/landeco/research/leda/; Kleyer et al. 2011). Data on pollen dispersal (insect- or wind dispersal or selfing), seed dispersal (zoochory= Zoo, anemochory= anemo, hydrochory=hydro, hemerochory=hemero and autochory=autochor) and life strategies (C=Competitor, R=Ruderal, S=Stress-tolerant) were based on BIOLFLOR (http://www2.ufz.de/biolflor/), or Rothmaler (2011) and completed with the 3D Dispersal Diaspore Database (Hintze et. al 2013; www.seed-dispersal.info/terms-of-use.html) considering indices ranks > 0.5. SC = SC; SI = SI.

Species	**Family**	**Type of kettle hole**	**Self-compatibility**	**Pollen vector**	**Seed dispersal**	**Life span**	**Seed bank longevity**	**Life strategies**
Acer platanoides L.	Sapindaceae	Permanent	SI	insect	Anemo	long	0	C
Acer pseudoplatanus L.	Sapindaceae	Both	Both	insect	Anemo	long	0	C
Achillea millefolium L.	Asteraceae	Both	SI	insect	Zoo-Anemo-Auto	long	0.1	C
Acorus calamus L.	Acoraceae	Permanent	NA	NA	Hydro-Hemero	long	0	CS
Aegopodium podagraria L.	Apiaceae	Permanent	SC	insect	Zoo-Anemo	long	0.26	C
Aethusa cynapium L.	Apiaceae	Permanent	SC	insect/selfing	Anemo-Hemero	short	0.97	CR
Agrostis capillaris L.	Poaceae	Permanent	Both	wind	Anemo-Hydro	long	0.52	CSR
Agrostis stolonifera L.	Poaceae	Both	Both	wind	Zoo-Anemo-Hydro	long	0.38	CSR
Alisma lanceolatum With.[ab]	Alismataceae	Ephemeral	SC	Insect/wind	Hydro	long	NA	CSR
Alisma plantago-aquatica L.[b]	Alismataceae	Both	Both	Insect/wind	Zoo-Hydro	long	0.84	CSR
Alliaria petiolata (M. Bieb.) Cavara & Grande	Brassicaceae	Permanent	SC	insect/selfing	Zoo-Anemo-Hemero-Auto	short/long	0.25	CR
Allium oleraceum L.	Amaryllidaceae	Permanent	NA	insect	Hemero	long	0	CSR
Allium vineale L.	Amaryllidaceae	Permanent	NA	insect	Hemero	long	0.08	C
Alopecurus aequalis Sobol.	Poaceae	Both	SC	wind/selfing	Zoo-Anemo-Hydro	short/long	0.67	SR
Alopecurus geniculatus L.	Poaceae	Both	SC	wind	Zoo-Anemo-Hydro	short/long	0.73	CSR
Alopecurus pratensis L.	Poaceae	Permanent	SI	wind	Zoo-Anemo-Hydro	long	0.08	C
Amaranthus retroflexus L.	Amaranthaceae	Ephemeral	SC	wind/selfing	Zoo-Hemero	short	0.79	CR
Anagallis arvensis L.	Primulaceae	Both	SC	insect/selfing	Hemero	short	0.82	R
Anchusa arvensis (L.) M.Bieb.	Boraginaceae	Both	NA	insect/selfing	Zoo-Hemero-Auto	short/long	0.27	CR
Anthriscus sylvestris (L.) Hoffm.	Apiaceae	Permanent	SC	insect/selfing	Auto	short/long	0.12	C
Apera spica-venti (L.) P. Beauv.	Poaceae	Both	Both	wind	Zoo-Anemo-Hydro	short	0.53	CR
Arctium lappa L.	Asteraceae	Both	SC	insect/selfing	Zoo	short/long	0.75	C
Arctium minus (Hill) Bernh.	Asteraceae	Both	SC	insect/selfing	Zoo	short/long	0.67	C
Arctium tomentosum Mill.	Asteraceae	Both	SC	insect/selfing	Zoo-Anemo	short/long	0	C
Armoracia rusticana P. Gaertn., B.Mey. & Scherb.	Brassicaceae	Both	SI	insect/selfing	Hemero	long	0	C
Arrhenatherum elatius (L.) P. Beauv. ex J. Presl & C. Presl[b]	Poaceae	Permanent	Both	wind/selfing	Zoo-Anemo-Hydro	long	0.06	C
Artemisia vulgaris L.	Asteraceae	Both	NA	wind	Zoo-Hemero	long	0.34	C
Athyrium filix-femina (L.) Roth	Woodsiaceae	Permanent	Both	NA	Anemo	long	NA	CS

Atriplex patula L.	Amaranthaceae	Both	SC	insect/wind/selfing	Hydro-Hemero	short	0.52	CR
Ballota nigra L.	Lamiaceae	Permanent	SC	insect/selfing	Zoo	long	0.63	C
Barbarea vulgaris R.Br.	Brassicaceae	Permanent	Both	insect/selfing	Hydro-Hemero-Auto	short/long	0.31	CR
Bidens tripartita L. [b]	Asteraceae	Both	SC	insect/selfing	Zoo-Hydro	short	0.4	CR
Bromus hordeaceus L.	Poaceae	Both	SC	wind/selfing	Anemo	short/long	0.13	CR
Bromus inermis Leyss.	Poaceae	Permanent	Both	wind	Anemo-Hydro	long	0	C
Bromus sterilis L.	Poaceae	Both	SC	selfing	Zoo-Anemo	short/long	0	CR
Bryonia alba L.	Cucurbitaceae	Permanent	SC	insect	Zoo-Hemero	long	NA	C
Calamagrostis canescens (Weber) Roth	Poaceae	Permanent	SI	wind	Zoo-Anemo	long	0.23	CS
Calamagrostis epigejos (L.) Roth	Poaceae	Both	SI	wind	Zoo-Anemo-Hydro	long	0.14	C
Capsella bursa-pastoris (L.) Medik.	Brassicaceae	Both	SC	insect/selfing	Zoo-Hydro-Hemero-Auto	short	0.65	R
Carduus crispus Guirão ex Nyman	Asteraceae	Both	SC	insect/selfing	Anemo	short/long	0.29	CR
Carex acuta L.	Cyperaceae	Permanent	SI	wind	Anemo-Hydro	long	0.26	CS
Carex acutiformis Ehrh.	Cyperaceae	Permanent	SC	wind	Hydro	long	0.09	CS
Carex hirta L.	Cyperaceae	Permanent	SC	wind	Zoo-Anemo	long	0.19	C
Carex pseudocyperus L.	Cyperaceae	Permanent	SC	wind	Hydro	long	0.5	CS
Carex remota L. [a]	Cyperaceae	Permanent	SC	wind	Hydro	long	0.83	CS
Carex riparia Curtis	Cyperaceae	Both	SC	wind	Hydro	long	0.15	CS
Carex vulpina L. [a]	Cyperaceae	Permanent	SC	wind	Zoo-Anemo-Hydro	long	0.83	CSR
Carpinus betulus L.	Betulaceae	Ephemeral	NA	wind	Anemo-Other	long	0	C
Centaurea cyanus L.	Asteraceae	Both	NA	insect	Zoo-Anemo	short	0.6	CR
Cerastium fontanum supsp. *vulgare* (Hartm.) Greuter & Burdet	Caryophyllaceae	Permanent	SC	insect/selfing	Zoo-Hemero	short/long	0.21	CR
Cerastium semidecandrum L.	Caryophyllaceae	Permanent	SC	insect/selfing	Zoo-Anemo	short	0.21	R
Prunus avium L.	Rosaceae	Permanent	SI	insect/selfing	Zoo-Hemero	long	0	C
Ceratophyllum demersum L. [a]	Ceratophyllaceae	Both	SC	water	Hydro	long	0	NA
Ceratophyllum submersum L.	Ceratophyllaceae	Both	SC	water	Zoo-Hydro	long	NA	NA
Chaerophyllum temulum L.	Apiaceae	Permanent	SC	insect	Auto	long	NA	CR
Chenopodium album L. [b]	Amaranthaceae	Both	SC	wind	Zoo-Hydro-Hemero	short	0.83	CR
Chenopodium polyspermum L.	Amaranthaceae	Both	SC	wind	Zoo-Hemero	short	0.76	CR
Chenopodium rubrum L.	Amaranthaceae	Permanent	SC	insect/wind/selfing	Hydro-Hemero	long	0.81	CR
Circaea lutetiana L.	Onagraceae	Permanent	SC	insect/selfing	Zoo	long	0	CS
Cirsium arvense (L.) Scop.	Asteraceae	Both	SC	insect	Hydro	long	0.2	C
Cirsium vulgare (Savi) Ten.	Asteraceae	Both	SC	insect	Hydro	short/long	0.2	CR
Clinopodium vulgare L.	Lamiaceae	Permanent	SC	insect	Zoo-Auto	long	0.44	CS
Convolvulus arvensis L.	Convolvulaceae	Both	Both	insect/selfing	Zoo-Hemero	long	0.1	CR
Conyza canadensis (L.) Cronquist	Asteraceae	Both	NA	insect/selfing	Anemo	short/long	0.5	CR
Crataegus monogyna Jacq.	Rosaceae	Permanent	Both	insect/selfing	Zoo	long	0.03	C
Cuscuta europaea L.	Convolvulaceae	Permanent	SC	selfing	Zoo-Anemo	short	NA	NA
Cynoglossum officinale L.	Boraginaceae	Permanent	SC	insect/selfing	Zoo	short/long	0.11	CS
Dactylis glomerata L.	Poaceae	Both	Both	wind/selfing	Zoo-Anemo-Hydro	long	0.15	C

Daucus carota L.	Apiaceae	Both	SC	insect/selfing	Zoo-Hemero	short/long	0.31	CR
Descurainia sophia (L.) Webb ex Prantl	Brassicaceae	Permanent	SC	insect/selfing	Zoo-Anemo-Auto	short	0.86	CR
Dipsacus fullonium L.	Caprifoliaceae	Permanent	Both	insect/selfing	Hemero	short/long	0.06	CR
Dryopteris filix-mas(L.) Schott	Dryopteridaceae	Permanent	SC	NA	Anemo	long	NA	CS
Echinochloa crus-galli (L.) P.Beauv. [b]	Poaceae	Both	SC	wind/selfing	Zoo	short	0.7	CR
Eleocharis palustris (L.) Roem. & Schult.	Cyperaceae	Both	SC	wind	Zoo-Anemo	short/long	0.22	CS
Elymus repens (L.) Gould	Poaceae	Both	Both	wind	Zoo-Anemo	long	0.07	C
Epilobium hirsutum L.	Onagraceae	Both	SC	insect/selfing	Anemo	long	0.64	C
Epilobium obscurum (Schreb.) Schreb. [a]	Onagraceae	Permanent	SC	selfing	Anemo	long	0.75	CS
Epilobium palustre L. [a]	Onagraceae	Both	SC	insect/selfing	Anemo-Hydro	long	0.25	CSR
Epilobium parviflorum Schreb.	Onagraceae	Both	SC	insect/selfing	Anemo	long	0.06	CS
Epilobium roseum (Schreb.) Schreb.	Onagraceae	Permanent	SC	insect/selfing	Anemo	long	0	CS
Epipactis helleborine (L.) Crantz	Orchidaceae	Permanent	SC	insect/selfing	Zoo-Anemo	long	0	CSR
Equisetum arvense L.	Equisetaceae	Both	SC	NA	Anemo	long	NA	CR
Equisetum palustre L.	Equisetaceae	Both	SI	NA	Anemo-Hydro	long	NA	CSR
Erysimum cheiranthoides L.	Brassicaceae	Both	SC	insect/selfing	Anemo-Auto	short	0.58	CR
Euonymus europaeus L.	Celastraceae	Permanent	NA	insect	Zoo	long	0	C
Euphorbia exigua L. [a]	Euphorbiaceae	Ephemeral	SC	insect	Hemero	short/long	0.85	R
Euphorbia helioscopia L.	Euphorbiaceae	Both	SC	insect/selfing	Hemero-Auto	short	0.63	R
Fagopyrum tataricum (L.) Gaertn.	Polygonaceae	Permanent	SC	insect/wind/selfing	Hemero	short	NA	CR
Falcaria vulgaris Bernh.	Apiaceae	Permanent	SC	insect	Anemo-Hemero	short/long	0	CS
Fallopia convolvulus (L.) . L ve	Polygonaceae	Both	SC	insect/selfing	Zoo-Hemero	short	0.63	CR
Festuca gigantea (L.) Vill.	Poaceae	Permanent	SC	wind	Zoo-Anemo	long	0.08	CS
Festuca rubra L.	Poaceae	Permanent	Both	wind	Zoo-Anemo-Hydro	short/long	0.14	C
Fraxinus excelsior L.	Oleaceae	Permanent	Both	wind	Anemo	long	0.02	C
Fumaria officinalis L. [b]	Papaveraceae	Ephemeral	SC	insect/selfing	Zoo-Hemero	short	0.61	R
Galeopsis bifida Boenn.	Lamiaceae	Permanent	SC	insect/selfing	Zoo-Hydro	short	NA	CR
Galeopsis speciosa Mill.	Lamiaceae	Permanent	SC	insect/selfing	Zoo	short	0	CR
Galeopsis tetrahit L.	Lamiaceae	Both	SC	insect/selfing	Zoo	short	0.45	CR
Galeopsis tetrahit x bifida	Lamiaceae	Permanent	NA	NA	Zoo	short	NA	CR
Galium aparine L.	Rubiaceae	Both	SC	insect/selfing	Zoo-Hemero	short	0.16	CR
Galium mollugo L.	Rubiaceae	Permanent	Both	insect/selfing	Zoo	long	0.1	C
Galium palustre L. [a]	Rubiaceae	Permanent	SC	insect/selfing	Hydro	long	0.18	CS
Genista tinctoria L.	Fabaceae	Permanent	SI	insect	Auto	long	0	CS
Geranium dissectum L. [a]	Geraniaceae	Permanent	SC	insect/selfing	Hemero-Auto	short/long	0.21	CR
Geranium pusillum L.	Geraniaceae	Both	SC	insect/selfing	Zoo-Hemero	short/long	0.38	C
Geranium robertianum L.	Geraniaceae	Permanent	SC	insect/selfing	Zoo	short/long	0.35	CSR
Geum urbanum L.	Rosaceae	Both	SC	insect/selfing	Zoo	long	0.05	CSR

Glechoma hederacea L.	Lamiaceae	Permanent	Both	insect	Zoo	long	0.2	CSR
Glyceria fluitans (L.) R. Br.	Poaceae	Both	Both	wind	Zoo-Hydro	long	0.61	CS
Glyceria maxima (Hartm.) Holmb.	Poaceae	Permanent	NA	wind	Zoo-Hydro	long	0.17	CS
Glyceria notata Chevall. [a]	Poaceae	Both	SC	selfing	Zoo-Hydro	long	1	CS
Gnaphalium sylvaticum L.	Asteraceae	Permanent	SC	insect/selfing	Anemo	long	0.71	CSR
Gnaphalium uliginosum L. [b]	Asteraceae	Both	SC	insect/selfing	Zoo-Hydro	short	0.89	R
Heracleum mantegazzianum Sommier & Levier	Apiaceae	Ephemeral	SC	insect/selfing	Anemo-Hemero	short/long	0	C
Heracleum sphondylium L.	Apiaceae	Permanent	SC	insect/selfing	Zoo-Anemo-Hydro	short/long	0.04	C
Holcus lanatus L.	Poaceae	Permanent	Both	wind/selfing	Zoo-Anemo-Hydro	long	0.51	C
Holcus mollis L.	Poaceae	Permanent	Both	wind	Zoo-Anemo-Hydro	long	0.03	CSR
Hottonia palustris L. [a]	Primulaceae	Permanent	Both	insect	Zoo-Hydro	long	NA	NA
Humulus lupulus L.	Cannabaceae	Permanent	SI	wind	Anemo	long	0.25	C
Hypericum perforatum L.	Hypericaceae	Both	SC	insect/selfing	Zoo-Auto	long	0.64	C
Impatiens glandulifera Royle	Balsaminaceae	Permanent	SC	insect	Auto	short	0	CR
Impatiens parviflora DC.	Balsaminaceae	Permanent	SC	insect/selfing	Auto	short	0	SR
Iris pseudacorus L.	Iridaceae	Both	Both	insect	Zoo-Hydro-Hemero	long	0	CS
Juncus articulatus L.	Juncaceae	Both	SC	wind	Zoo-Auto	long	0.91	CSR
Juncus bufonius L. [b]	Juncaceae	Both	SC	wind/selfing	Zoo	short	0.9	R
Juncus effusus L.	Juncaceae	Both	SC	insect/wind/selfing	Zoo-Auto	long	0.93	C
Juncus inflexus L. [a]	Juncaceae	Permanent	SC	wind/selfing	Anemo	long	0.63	C
Lactuca serriola L.	Asteraceae	Both	SC	insect/selfing	Anemo	short/long	0.21	CR
Lamium purpureum L.	Lamiaceae	Both	NA	insect/selfing	Zoo-Anemo	short/long	0.59	R
Lapsana communis L.	Asteraceae	Permanent	SC	insect/selfing	Zoo-Hemero-Auto	short/long	0.55	CR
Lathyrus pratensis L.	Fabaceae	Permanent	Both	insect	Zoo-Auto	long	0.05	C
Lemna gibba L.	Araceae	Permanent	SC	wind/water/selfing/insect	Zoo-Hydro	NA	NA	NA
Lemna minor L.	Araceae	Both	Both	wind/water/selfing/insect	Zoo-Hydro	NA	0	NA
Lemna trisulca L.	Araceae	Both	SC	wind/water/selfing/insect	Zoo-Hydro	NA	0	NA
Leontodon autumnalis L.	Asteraceae	Permanent	Both	insect	Zoo-Anemo	long	0.11	CSR
Lepidium ruderale L.	Brassicaceae	Permanent	SC	selfing	Zoo-Anemo-Hemero	short	0	R
Leucanthemum vulgare (Vaill.) Lam. [a]	Asteraceae	Permanent	SC	insect/selfing	Zoo-Anemo-Hemero	long	0.36	C
Lolium multiflorum Lam.	Poaceae	Both	SI	wind	Zoo-Anemo-Hydro	short/long	0.33	C
Lolium perenne L.	Poaceae	Ephemeral	Both	wind	Zoo-Anemo-Hydro	short/long	0.19	C
Lycopus europaeus L.	Lamiaceae	Both	SC	insect	Zoo-Hydro	long	0.19	CS
Lysimachia nummularia L.	Primulaceae	Permanent	Both	insect	Hydro-Hemero	long	0.08	CSR
Lysimachia vulgaris L.	Primulaceae	Permanent	SI	insect/selfing	Hydro-Auto	long	0.29	CS
Lythrum salicaria L.	Lythraceae	Both	SI	insect/selfing	Zoo	long	0.36	CS
Malus domestica Borkh.	Rosaceae	Permanent	Both	insect	Zoo	long	0	C
Matricaria discoidea DC.	Asteraceae	Both	SC	insect/selfing	Zoo-Hemero	short	0.86	R
Matricaria recutita L.	Asteraceae	Both	NA	insect	Zoo-Hemero	short/long	0.72	R
Medicago lupulina L.	Fabaceae	Both	SC	insect/selfing	Zoo-Anemo-Hemero	short/long	0.35	CSR

Melica uniflora Retz. [a]	Poaceae	Permanent	NA	wind	Zoo	long	0	C
Melilotus albus Medik.	Fabaceae	Permanent	Both	insect/selfing	Zoo-Anemo	short/long	0.05	CR
Mentha aquatica L.	Lamiaceae	Permanent	SC	insect	Hydro-Auto	long	0.51	CS
Mentha arvensis L.	Lamiaceae	Both	SC	insect	Zoo-Hydro-Hemero	long	0.28	C
Chaenorhinum minus (L.) Lange	Plantaginaceae	Ephemeral	SC	selfing	Anemo	NA	NA	R
Milium effusum L.	Poaceae	Permanent	SC	wind/selfing	Zoo	long	0.53	CS
Moehringia trinervia (L.) Clairv.	Caryophyllaceae	Permanent	SC	insect/selfing	Zoo	short/long	0.59	CSR
Myosotis arvensis (L.) Hill	Boraginaceae	Both	SC	insect/selfing	Zoo-Hemero	short/long	0.44	R
Myosurus minimus L. [a]	Ranunculaceae	Ephemeral	SC	insect/selfing	Zoo-Anemo	short/long	1	CS
Oenanthe aquatica (L.) Poir.	Apiaceae	Both	SC	insect/selfing	Zoo-Hydro	short/long	0	R
Papaver rhoeas L.	Papaveraceae	Both	SI	insect	Anemo-Auto	short/long	0.63	CS
Lythrum portula (l.) D.A. Webb [b]	Lythraceae	Both	SC	selfing	Zoo-Hydro	NA	1	CSR
Petasites hybridus (L.) G. Gaertn., B. Mey. & Scherb. [b]	Asteraceae	Ephemeral	SI	insect	Zoo-Anemo-Hydro	long	0	CR
Phacelia tanacetifolia Benth.	Boraginaceae	Permanent	SC	insect/selfing	NA	short	NA	S
Phalaris arundinacea L.	Poaceae	Both	SI	wind/selfing	Zoo-Anemo-Hydro	long	0.06	CS
Phleum pratense L.	Poaceae	Both	Both	insect/wind/selfing	Zoo-Anemo	long	0.2	C
Phragmites australis (Cav.) Trin. ex Steud.	Poaceae	Permanent	Both	wind	Hydro	long	0.02	C
Picris hieracioides Sibth. & Sm.	Asteraceae	Both	SI	insect/selfing	Anemo	short/long	0.18	C
Plantago lanceolata L.	Plantaginaceae	Both	Both	insect/wind/selfing	Zoo-Auto	long	0.24	CS
Plantago major subsp. *intermedia* (Gilib.) Lange	Plantaginaceae	Both	SC	wind/selfing	NA	NA	NA	CSR
Poa annua L.	Poaceae	Both	SC	wind/selfing	Zoo-Anemo	short	0.83	S
Poa nemoralis L.	Poaceae	Permanent	SC	wind	Zoo-Anemo	long	0.39	CS
Poa palustris L.	Poaceae	Both	SC	wind	Zoo-Anemo-Hydro	long	0.11	C
Poa trivialis L.	Poaceae	Both	SC	wind	Zoo-Anemo-Hydro	NA	0.59	R
Polygonum amphibium L.	Polygonaceae	Both	SI	insect	Zoo-Hydro	long	0	R
Polygonum aviculare L. [b]	Polygonaceae	Both	SC	insect/selfing	Zoo-Hemero	short	0.57	CR
Polygonum hydropiper L.	Polygonaceae	Both	SC	selfing	Zoo-Hydro-Hemero	short	0.4	CS
Polygonum lapathifolium L.	Polygonaceae	Both	SC	insect/selfing	Zoo-Hydro-Hemero	short	0.69	R
Polygonum minus Huds.	Polygonaceae	Both	SC	insect/selfing	Hydro	short	0.25	CR
Polygonum persicaria L.	Polygonaceae	Both	NA	NA	Zoo-Hydro-Hemero	short	0.69	CS
Populus tremula L.	Salicaceae	Permanent	SI	wind	Anemo	long	0	CR
Potamogeton natans L.	Potamogetonaceae	Ephemeral	SC	wind	Zoo	NA	NA	C
Potentilla anserina L.	Rosaceae	Permanent	SI	insect	Zoo-Hydro-Hemero	long	0.17	NA
Potentilla reptans L.	Rosaceae	Both	NA	insect	Zoo-Hydro	long	0.33	S
Prunus cerasifera Ehrh.	Rosaceae	Permanent	Both	insect	Hemero	NA	0	CS
Prunus domestica L.	Rosaceae	Permanent	Both	insect/selfing	Hemero	long	0	CS
Prunus spinosa L.	Rosaceae	Permanent	NA	insect/selfing	Zoo-Hemero	long	0	C
Quercus petraea (Matt.) Liebl.	Fagaceae	Permanent	SI	wind	Other	long	0	CSR
Quercus robur L.	Fagaceae	Permanent	Both	wind	Other	long	0	C

Ranunculus repens L.	Ranunculaceae	Both	Both	insect/selfing	Zoo-Hemero-Auto	long	0.54	C
Ranunculus sceleratus L. [b]	Ranunculaceae	Both	SI	insect	Zoo-Hydro	short	0.79	SR
Raphanus raphanistrum L.	Brassicaceae	Permanent	SI	insect/selfing	Zoo-Anemo-Hydro-Hemero	short/long	0.3	CR
Reynoutria sachalinensis (F. Schmidt) Nakai	Polygonaceae	Permanent	SI	insect	Anemo-Hydro-Hemero	long	NA	CR
Ribes uva-crispa L.	Grossulariaceae	Permanent	NA	insect/selfing	Auto	long	0	C
Elymus caninus (L.) L. [a]	Poaceae	Permanent	SC	wind/selfing	Zoo-Anemo-Hydro	long	0.67	CS
Rorippa amphibia (L.) Besser	Brassicaceae	Both	SI	insect/selfing	Zoo-Hydro	long	0.25	CS
Rorippa palustris (L.) Besser [b]	Brassicaceae	Both	SC	insect/selfing	Zoo-Hydro	short/long	0.63	CR
Rosa spec.	Rosaceae	Permanent	NA	NA	Zoo-Hemero-Auto	long	0.04	C
Rubus caesius L.	Rosaceae	Permanent	SC	insect/selfing	Zoo	long	0	C
Rubus idaeus L.	Rosaceae	Permanent	SI	insect/selfing	Auto	long	0.68	C
Rubus spec.	Rosaceae	Permanent	NA	NA	Hemero-Auto	long	NA	C
Rumex crispus L.	Polygonaceae	Both	Both	wind	Zoo-Anemo	short/long	0.3	NA
Rumex maritimus L. [b]	Polygonaceae	Both	SC	wind/selfing	Zoo-Anemo-Hydro	short/long	0.95	SR
Rumex obtusifolius L.	Polygonaceae	Both	Both	wind	Zoo-Anemo-Hydro	long	0.54	SR
Rumex patientia L.	Polygonaceae	Both	NA	wind	NA	NA	0	C
Rumex sanguineus L. [a]	Polygonaceae	Both	NA	wind	Zoo	long	0.83	CS
Rumex stenophyllus Ledeb.	Polygonaceae	Ephemeral	NA	wind	NA	NA	NA	C
Salix alba L. [a]	Salicaceae	Both	SI	insect	Anemo-Hydro	long	0.44	R
Salix caprea L.	Salicaceae	Permanent	SI	insect	Anemo-Hydro	long	0.02	C
Salix cinerea L.	Salicaceae	Both	SI	insect	Anemo-Hydro	long	0	C
Salix silesiaca Willd.	Salicaceae	Permanent	SI	insect	Anemo-Hydro	long	NA	C
Salix viminalis L.	Salicaceae	Both	SI	insect	Anemo-Hydro	NA	0	SR
Sambucus nigra L.	Adoxaceae	Both	SC	insect/selfing	Hemero	long	0.22	C
Schoenoplectus lacustris (L.) Palla	Cyperaceae	Permanent	SC	Insect/wind	Zoo	NA	0	CSR
Scrophularia nodosa L.	Scrophulariaceae	Permanent	SC	insect	Auto	long	0.9	CS
Scrophularia umbrosa Dumort.	Scrophulariaceae	Permanent	NA	insect/selfing	Hydro-Auto	long	1	C
Scutellaria galericulata L.	Lamiaceae	Permanent	SC	insect/selfing	Hydro-Auto	long	0	CSR
Senecio vulgaris L.	Asteraceae	Ephemeral	Both	insect/selfing	Zoo-Hydro	short/long	0.55	C
Setaria viridis (L.) P. Beauv.	Poaceae	Ephemeral	SC	wind	Zoo	short	0.77	CS
Silene latifolia Poir.	Caryophyllaceae	Permanent	SC	insect	Hemero-Auto	NA	0.44	CR
Silene noctiflora L. [a]	Caryophyllaceae	Both	SC	insect/selfing	Hemero-Auto	short	1	R
Sinapis arvensis L.	Brassicaceae	Permanent	SI	insect/selfing	Zoo-Hemero-Auto	short	0.83	S
Sisymbrium officinale (L.) Scop.	Brassicaceae	Both	SC	insect/selfing	Hemero-Auto	short	0.41	R
Solanum dulcamara L.	Solanaceae	Both	SC	insect	Zoo	long	0.22	SR
Sonchus arvensis L.	Asteraceae	Both	SI	insect/selfing	Anemo	long	0.78	R
Sonchus asper (L.) Hill	Asteraceae	Both	SC	insect/selfing	Zoo-Anemo	short	0.51	CS
Sonchus oleraceus L.	Asteraceae	Permanent	SC	selfing	Zoo-Anemo	short	0.75	C
Sorbus aucuparia L.	Rosaceae	Permanent	Both	insect	Zoo-Other	long	0.02	C
Sparganium emersum Rehmann [a]	Typhaceae	Ephemeral	SC	wind	Anemo	NA	NA	C
Sparganium erectum L.	Typhaceae	Both	SC	Insect/wind	Zoo-Hydro	long	0	SR

Spergularia rubra (L.) J. Presl & C. Presl	Caryophyllaceae	Ephemeral	SC	insect/selfing	Zoo	short/long	1	SR
Spirodela polyrhiza (L.) Schleid. [b]	Araceae	Both	SC	wind/water/selfing/insect	Zoo-Hydro	NA	NA	C
Stachys palustris L.	Lamiaceae	Permanent	SC	insect/selfing	Hydro-Auto	long	0.04	NA
Stellaria aquatica (L.) Scop. [b]	Caryophyllaceae	Permanent	SC	insect/selfing	Zoo-Anemo-Hydro	long	0	C
Stellaria media (L.) Vill.	Caryophyllaceae	Both	Both	insect/selfing	Zoo-Hydro-Hemero	short/long	0.71	R
Stellaria palustris Ehrh. ex Retz.	Caryophyllaceae	Permanent	SC	insect/selfing	Hydro	long	0.13	CS
Symphytum officinale L.	Boraginaceae	Permanent	NA	insect/selfing	Zoo-Hydro-Hemero	long	0	C
Tanacetum vulgare L.	Asteraceae	Ephemeral	NA	insect	Zoo-Hydro-Hemero	long	0.16	CSR
Taraxacum spec.	Asteraceae	Both	NA	NA	Zoo-Anemo	NA	0.26	CS
Thlaspi arvense L. [b]	Brassicaceae	Both	SC	insect/selfing	Zoo-Anemo-Hemero-Auto	short	0.87	CS
Torilis japonica (Houtt.) DC.	Apiaceae	Permanent	SC	insect	Zoo	short/long	0.47	CSR
Trifolium hybridum L.	Fabaceae	Both	SI	insect	Zoo	long	0.5	R
Trifolium incarnatum L.	Fabaceae	Permanent	NA	insect/selfing	Zoo-Anemo	NA	1	R
Trifolium pratense L.	Fabaceae	Permanent	SI	insect	Zoo	long	0.24	SR
Trifolium repens L.	Fabaceae	Both	Both	insect	Zoo	long	0.38	C
Tripleurospermum perforatum (Mérat) Laínz	Asteraceae	Both	Both	insect	Zoo-Hemero	NA	0.74	CSR
Typha latifolia L.	Typhaceae	Both	SC	wind	Anemo-Hydro	long	0.58	CS
Urtica dioica L. [b]	Urticaceae	Both	SC	Insect/wind	Anemo-Hydro-Auto	long	0.65	R
Veronica anagallis-aquatica L. [a]	Plantaginaceae	Both	SC	insect/selfing	Zoo	long	0.67	R
Veronica chamaedrys L.	Plantaginaceae	Permanent	NA	selfing	Zoo-Hydro	long	0.42	S
Veronica persica Poir. L.	Plantaginaceae	Both	SC	insect/selfing	Hemero	short/long	0.66	CSR
Veronica serpyllifolia L.	Plantaginaceae	Ephemeral	SC	insect	Zoo	short/long	0.71	CS
Vicia sativa subsp. nigra (L.) Ehrh.	Fabaceae	Both	SC	insect/selfing	Auto	short/long	0	C
Vicia cracca L.	Fabaceae	Permanent	SC	insect	Zoo-Auto	long	0.05	C
Vicia hirsuta (L.) Gray	Fabaceae	Both	SC	insect/selfing	Zoo-Auto	short/long	0.25	CR
Vicia tetrasperma (L.) Schreb.	Fabaceae	Permanent	SC	insect/selfing	Zoo-Auto	short/long	0.2	CS
Vicia villosa Roth	Fabaceae	Both	NA	insect	Hemero	short/long	0.18	CSR
Viola arvensis Murray	Violaceae	Both	SC	insect/selfing	Auto	short	0.53	S

[a] Plant species present in the state red-list of Brandenburg (Ristow et al. 2006).

[b] Plant species from seed banks were found.

Table A3: List of species with seeds were found in the seedbank in a subset of 20 kettle holes.

Species	# individuals in flat ephemeral ponds	# individuals in steep permanent ponds
Alisma plantago-aquatica L.	1	23
Alisma lanceolatum With.	0	1
Arrhenatherum elatius L.	2	3
Bidens tripartita L.	112	57
Brassica napus L.	0	5
Brassica spec. 1	8	16
Brassica spec. 2	60	2
Brassica spec. 3	0	13
Chenopodium album L.	335	21
Echinochloa curs-galli (L.) P. Beauv.	15	7
Epilobium spec.	49	6
Fumaria officinalis L.	5	10
Geranium spec.	0	5
Gnaphalium uliginosum L.	12	2
Juncus bufonius L.	2653	1255
Juncus spec. 1	167	142
Juncus spec. 2	34	99
Juncus spec. 3	0	3
Lythrum portula (L.) D.A.Webb	64	0
Matricaria spec.	377	264
Myosotis spec.	0	1
Oenanthe spec.	0	73
Petasites hybridus (L.) G. Gaerth, B. Mey. & Scherb	77	0
Polygonum spec.	0	9
Polygonum aviculare L.	1868	198
Ranunculus sceleratus L.	28	135
Rorippa palustris (L.) Besser	153	32
Rumex maritimus L.	100	0
Spirodela polyrhiza (L.) Schleid.	27	53
Stellaria aquatica (L.) Scop.	134	41
Thlaspi arvense L.	0	41
Urtica dioica L.	104	901
Unknown species x	1	0
Unknown species y	146	31
Total # individuals	**6532**	**3449**
Average number	**192.12**	**101.44**
Standard Error	**92.83**	**44.4**
N species	**25**	**30**
N species only (Both=21)	**4**	**9**

Table A4: Detailed Generalized Linear Models with a quasi-poisson distribution selection based on Explanatory Deviance and qAIC of germination in relation with type of kettle hole and treatment for the seed bank experiment and for species number in relation with area (log) and degree of spatial isolation for the entire community and for wetlands specialized species.

Seed bank experiment

Model	**glmID**	**ExplDev**	**adjExplDev**	**qAICc**	**dqAICc**	**df**	**weight**	**Model description**
1	**m1qp**	**0.025556**	**-0.02858**	**222.35**	**0**	**2**	**0.463503**	**Germination ~ Type**
2	m2qp	0.005269	-0.04999	226.74	4.390183	2	0.05161	Germination ~ Treatment
3	m3qp	0.030825	-0.0832	223.24	0.889648	3	0.297076	Germination ~ Type + Treatment
4	m4qp	0.030853	-0.15086	225.27	2.920979	4	0.107589	Germination ~ Type * Treatment
5	m5qp	0	0	225.86	3.508039	1	0.080222	Germination ~ 1

Entire community

Model	**glmID**	**ExplDev**	**adjExplDev**	**qAICc**	**dqAICc**	**df**	**weight**	**Model description**
1	m1qp	0.0991	0.0786	222	54.5	2	<0.001	All_sp ~ Freq
2	m2qp	0.2846	0.2684	200.5	33	2	<0.001	All_sp ~ Area_ha_log
3	m3qp	0.2461	0.2289	205	37.5	2	<0.001	All_sp ~ Type
4	m4qp	0.3454	0.3150	195.9	28.4	3	<0.001	All_sp ~ Area_ha_log + Freq
5	m5qp	0.3488	0.3185	195.5	28	3	<0.001	All_sp ~ Freq + Type
6	m6qp	0.4695	0.4448	181.5	14	3	<0.001	All_sp ~ Area_ha_log + Type
7	m7qp	0.3679	0.3228	195.8	28.3	4	<0.001	All_sp ~ Area_ha_log * Freq
8	m8qp	0.4548	0.4159	185.7	18.3	4	<0.001	All_sp ~ Freq * Type
9	m9qp	0.4695	0.4316	184	16.6	4	<0.001	All_sp ~ Area_ha_log * Type
10	m10qp	0.5365	0.5034	176.3	8.8	4	0.0094	All_sp ~ Area_ha_log + Freq + Type
11	m11qp	0.5365	0.4913	178.9	11.5	5	0.0025	All_sp ~ Area_ha_log + Freq + Area_ha_log * Type
12	**m12qp**	**0.6356**	**0.6000**	**167.5**	**0**	**5**	**0.7654**	**All_sp ~ Area_ha_log + Freq * Type**
13	m13qp	0.5551	0.5117	176.8	9.3	5	0.0073	All_sp ~ Area_ha_log * Freq * Type
14	m14qp	0.6632	0.6113	170	2.5	7	0.2145	All_sp ~ Area_ha_log * Freq + Area * Type + Freq * Type
15	m15qp	0.0000	0.0000	231.1	63.7	1	<0.001	All_sp ~ 1

Wetland community

Model	glmID	ExplDev	adjExplDev	qAICc	dqAICc	df	weight	Model description
1	sp1qp	0.0749	0.0539	183.79	30.85	2	0.0000	Wet_sp ~ Freq
2	sp2qp	0.2872	0.2710	165.13	12.19	2	0.0021	Wet _sp ~ Area_ha_log
3	sp3qp	0.0597	0.0384	185.12	32.18	2	0.0000	Wet _sp ~ Type
4	sp4qp	0.3298	0.2986	163.79	10.85	3	0.0041	Wet _sp ~ Area_ha_log + Freq
5	sp5qp	0.1363	0.0962	180.80	27.86	3	0.0000	Wet _sp ~ Freq + Type
6	sp6qp	0.3174	0.2857	164.88	11.94	3	0.0024	Wet _sp ~ Area_ha_log + Type
7	sp7qp	0.3305	0.2827	166.26	13.32	4	0.0012	Wet _sp ~ Area_ha_log * Freq
8	sp8qp	0.3000	0.2500	168.94	16.00	4	0.0003	Wet _sp ~ Freq * Type
9	sp9qp	0.3224	0.2740	166.97	14.03	4	0.0008	Wet _sp ~ Area_ha_log * Type
10	sp10qp	0.3622	0.3166	163.47	10.53	4	0.0048	Wet _sp ~ Area_ha_log + Freq + Type
11	sp11qp	0.3670	0.3053	165.70	12.76	5	0.0016	Wet _sp ~ Area_ha_log + Freq + Area_ha_log * Type
12	**sp12qp**	**0.5122**	**0.4646**	**152.94**	**0.00**	**5**	**0.9229**	**Wet _sp ~ Area_ha_log + Freq * Type**
13	sp13qp	0.3626	0.3004	166.09	13.15	5	0.0013	Wet _sp ~ Area_ha_log * Freq * Type
14	sp14qp	0.5147	0.4401	158.45	5.51	7	0.0586	Wet _sp ~ Area_ha_log * Freq + Area * Type + Freq * Type
15	sp15qp	0.0000	0.0000	188.08	35.14	1	0.0000	Wet _sp ~ 1

Best Models

Seed bank: m1qp

glm.nb (Germination ~ Type, data=Seed Bank Data, family=”quasipoisson”))

All species: m12qp

glm.nb (All_sp ~ Area_ha_log + Freq * Type, data=SITES, family=”quasipoisson”))

Wetland species: sp12qp

glm.nb (Wet_sp ~ Area _ha_log + Freq * Type, data=SITES, family=”quasipoisson”))

Table A5: Best fitted Linear Models and ANOVAs of colonization and dispersal traits.

TRAIT	BEST FITTED MODEL		SIGNIFICANT FACTORS		INTERACTIONS
Seed Longevity Index	SLI.m08 <- lm(SLI_mean~Type_of_kettle_hole + Tree_presence + NEAR_X + Type_of_kettle_hole*Tree_presence + Type_of_kettle_hole*NEAR_X+ Tree_presence*NEAR_X + Type_of_kettle_hole*Tree_presence*NEAR_X)		**Type_of_kettle_hole** ***	Tree_presence**	NEAR_X**
$R^2 = 0.70$		$F_{1,40}$	91.3155	8.6062	9.8589
		p	7.04E-12	0.005523	0.003173
Short-lived	Short.m08 <- lm(Per_sp_Short~Type_of_kettle_hole + Tree_presence + N_sp + Type_of_kettle_hole*Tree_presence + Type_of_kettle_hole*N_sp + Tree_presence*NEAR_X + Type_of_kettle_hole*Tree_presence*N_sp)		**Type_of_kettle_hole*****	Tree_presence *	Type_of_kettle_hole:N_sp .
$R^2 = 0.65$		$F_{1,32}$	46.9606	6.4047	3.8207
		p	3.89E-08	0.01564	0.05801
Long-lived	Long.m08 <- lm(Per_sp_Long~Type_of_kettle_hole + Tree_presence + N_sp + Type_of_kettle_hole*Tree_presence + Type_of_kettle_hole*N_sp+ Tree_presence*NEAR_X + Type_of_kettle_hole*Tree_presence*N_sp)		**Type_of_kettle_hole** ***	Tree_presence *	N_sp *
$R^2 = 0.57$		$F_{1,38}$	61.3397	5.9637	5.2972
		p	1.90E-09	0.01937	0.02694
SC	SC.m06 <- lm(Per_sp_SC~Type_of_kettle_hole + N_sp)		Type_of_kettle_hole	N_sp *	
$R^2 = 0.19$		$F_{1,43}$	0	5.5361	
		p	0.99567	0.02327	
SI	SI.m04 <- lm(Per_sp_SI~Type_of_kettle_hole + Tree_presence)		**Type_of_kettle_hole** ***	Tree_presence *	
$R^2 = 0.26$		$F_{1,43}$	15.2226	5.5381	
		p	0.0003312	0.0232493	
Zoophily	In.pol.m08 <- lm(Per_sp_Zoophily~Type_of_kettle_hole + Tree_presence + N_sp + Type_of_kettle_hole*Tree_presence + Type_of_kettle_hole*N_sp + Tree_presence*NEAR_X + Type_of_kettle_hole*Tree_presence*N_sp)		**Type_of_kettle_hole** **	N_sp **	Type_of_kettle_hole:N_sp .
$R^2 = 0.31$		$F_{1,38}$	10.5447	8.6824	3.3277
		p	0.002437	0.005464	0.075993
Anemophily	Wind.pol.m08 <- lm(Per_sp_Anemophily~Type_of_kettle_hole + Tree_presence + N_sp+ Type_of_kettle_hole*Tree_presence + Type_of_kettle_hole*N_sp + Tree_presence*NEAR_X + Type_of_kettle_hole*Tree_presence*N_sp)		Type_of_kettle_hole	NEAR_X **	N_sp *
		$F_{1,38}$	0.0766	4.9158	9.123
$R^2 = 0.17$		p	0.783406	0.006835	0.032669
Hydrophily	Water.pol.m08 <- lm(Per_sp_Hydrophily~Type_of_kettle_hole + Tree_presence + N_sp + Type_of_kettle_hole*Tree_presence + Type_of_kettle_hole*N_sp + Tree_presence*NEAR_X + Type_of_kettle_hole*Tree_presence*N_sp)		**Type_of_kettle_hole** **	NEAR_X *	Type_of_kettle_hole:N_sp *
		$F_{1,38}$	11.5819	4.6384	4.7823
$R^2 = 0.24$		p	0.001582	0.037672	0.034979
Selfing	Other.pol.m02 <- lm(Per_sp_Other_pol~Tree_presence)		Type_of_kettle_hole		
$R^2 = 0.02$		$F_{1,38}$	1.6973		
		p	0.1994		
Zoochory	In.dis.m08 <- lm(Per_sp_Zoochory~Type_of_kettle_hole + Tree_presence + N_sp + Type_of_kettle_hole*Tree_presence + Type_of_kettle_hole*N_sp + Tree_presence*NEAR_X +Type_of_kettle_hole*Tree_presence*N_sp)		**Type_of_kettle_hole** **	NEAR_X **	
$R^2 = 0.29$		$F_{1,38}$	10.7986	7.321	
		p	0.00219	0.01015	
Anemochory	Wind.dis.m07 <- lm(Per_sp_Anemochory~Type_of_kettle_hole * N_sp)		**Type_of_kettle_hole** ***		Type_of_kettle_hole:N_sp *
$R^2 = 0.37$		$F_{1,38}$	23.213		4.8462
		p	1.92E-05		0.03325
Hydrochory	Water.dis.m08 <- lm(Per_sp_Hydrochory~Type_of_kettle_hole + Tree_presence + N_sp +Type_of_kettle_hole*Tree_presence + Type_of_kettle_hole*N_sp + Tree_presence*NEAR_X + Type_of_kettle_hole*Tree_presence*N_sp)		Type_of_kettle_hole	NEAR_X ***	
$R^2 = 0.25$		$F_{1,38}$	0.076	17.8137	
		p	0.784276	0.000146	
Hemerochory	Hemero.dis.m07 <- lm(Per_sp_Hemerochory~Type_of_kettle_hole * N_sp)		**Type_of_kettle_hole** ***		
$R^2 = 0.28$		$F_{1,42}$	16.5826		
		p	0.0002017		
Autochory	Auto.dis.m08 <- lm(Per_sp_Autochory~Type_of_kettle_hole + Tree_presence + N_sp + Type_of_kettle_hole*Tree_presence + Type_of_kettle_hole*N_sp + Tree_presence*NEAR_X + Type_of_kettle_hole*Tree_presence*N_sp)		**Type_of_kettle_hole** ***	NEAR_X *	
$R^2 = 0.35$		$F_{1,38}$	20.7001	5.769	
		p	5.36E-05	0.02131	

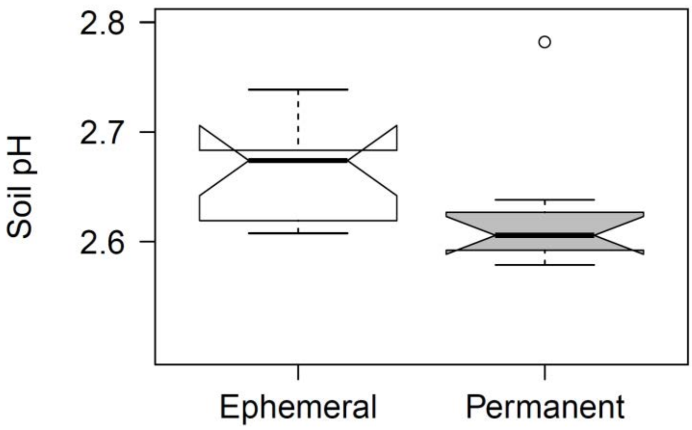


Fig. A1: Soil analysis of a subset of 20 kettle holes. Comparison pH between flat ephemeral and steep permanent kettle holes ($F_{1,18} = 3.71$ $P = 0.069$).

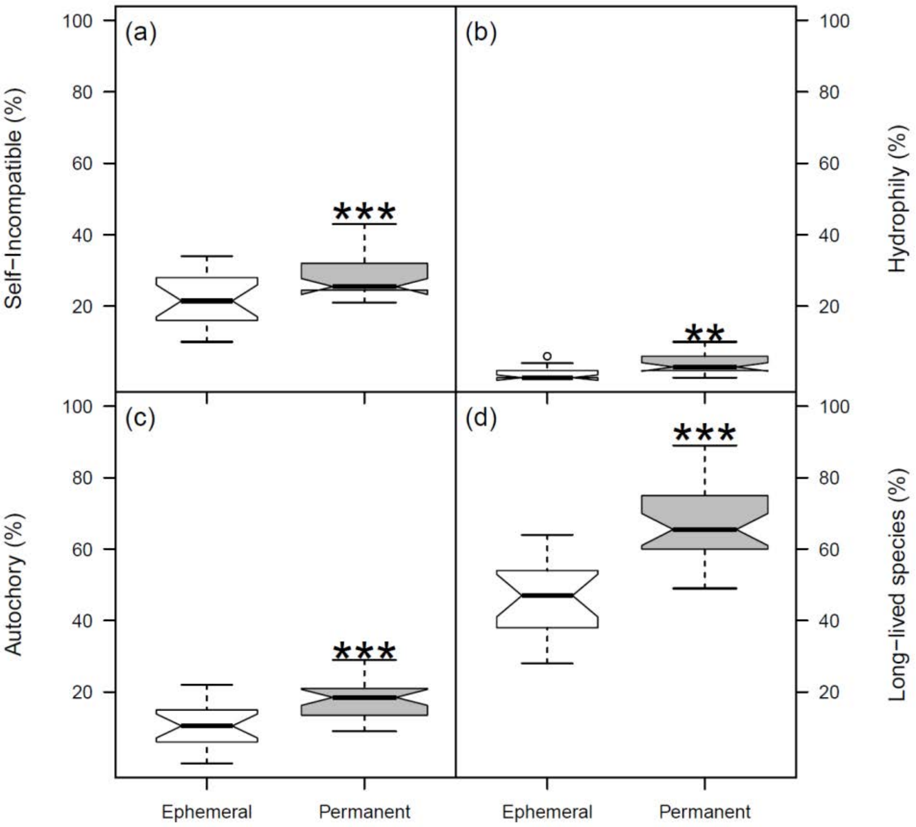


Fig. A2: Additional colonization and dispersal plant traits. Percentage of SI (A), hydrophilous (B), autochorous (C) and long-lived (D) species differ significantly (A: $F_{1,43} = 15.22$ $P < 0.001$; B: $F_{1,38} = 11.58$ $P < 0.01$; C: $F_{1,38} = 20.70$ $P < 0.001$; D: $F_{1,38} = 61.33$ $P < 0.001$ between flat ephemeral and steep permanent kettle holes.

APPENDIX B

Supplementary Material to Chapter 3

List of tables

List of figures

Table B1. List of plant species and bee species found in the kettle holes. Presence/absence of all plant species was recorded and identification was following Rothmaler (2011) and wild-bees specimens were collected using a color traps and later sent to Naturkunde Museum Berlin for identification.

	Plants				**Bees**	
1	*Acer*	*platanoides*	*Lolium*	*perenne*	*Andrena*	*anthrisci*
2	*Acer*	*pseudoplatanus*	*Lycopus*	*europaeus*	*Andrena*	*anthrisci cf.*
3	*Achillea*	*millefolium*	*Lysimachia*	*nummularia*	*Andrena*	*bicolor*
4	*Acorus*	*calamus*	*Lysimachia*	*vulgaris*	*Andrena*	*carantonica*
5	*Aegopodium*	*podagraria*	*Lythrum*	*salicaria*	*Andrena*	*chrysosceles*
6	*Aesculus*	*hippocastanum*	*Malus*	*sylvestris*	*Andrena*	*cineraria*
7	*Agrostis*	*stolonifera*	*Matricaria*	*chamomilla*	*Andrena*	*dorsata*
8	*Alisma*	*plantago-aquatica*	*Melica*	*uniflora*	*Andrena*	*flavipes*
9	*Alliaria*	*petiolata*	*Mentha*	*arvensis*	*Andrena*	*fucata*
10	*Allium*	*vineale*	*Millium*	*effusum*	*Andrena*	*fulva*
11	*Alnus*	*glutinosa*	*Moehringia*	*trinervia*	*Andrena*	*gravida*
12	*Alopecurus*	*aequalis*	*Myosotis*	*arvensis*	*Andrena*	*haemorrhoa*
13	*Alopecurus*	*geniculatus*	*Myosotis*	*scorpioides*	*Andrena*	*helvola*
14	*Anchusa*	*arvensis*	*Nymphea*	*alba*	*Andrena*	*helvola cf.*
15	*Anthriscus*	*sylvestris*	*Oenanthe*	*aquatica*	*Andrena*	*labiata*
16	*Apera*	*spica-venti*	*Papaver*	*rhoeas*	*Apis*	*mellifera*
17	*Arctium*	*sp.*	*Persicaria*	*amphibia*	*Andrena*	*minutula*
18	*Arrhenatherum*	*elatius*	*Persicaria*	*hydropiper*	*Andrena*	*minutuloides*
19	*Artemisia*	*vulgaris*	*Persicaria*	*lapitifolia*	*Andrena*	*nigroaenea*
20	*Athyrium*	*filix-femina*	*Persicaria*	*maculosa*	*Andrena*	*nitida*
21	*Ballota*	*nigra*	*Phalaris*	*arundinacea*	*Andrena*	*pilipes*
22	*Barbarea*	*vulgaris*	*Phleum*	*pratense*	*Andrena*	*praecox*
23	*Barbarea*	*vulgaris*	*Phragmites*	*australis*	*Andrena*	*strohmella*
24	*Betula*	*pendula*	*Picea*	*abies*	*Andrena*	*subopaca*
25	*Betula*	*pubescens*	*Plantago*	*lanceolata*	*Andrena*	*suerinensis*
26	*Bidens*	*cernua*	*Plantago*	*major*	*Andrena*	*tibialis*
27	*Bidens*	*frondosa*	*Plantago*	*media*	*Andrena*	*vaga*
28	*Bidens*	*tripartita*	*Poa*	*nemoralis*	*Andrena*	*ventralis*
29	*Bromus*	*hordeaceus*	*Poa*	*palustris*	*Andrena*	*wilkella*
30	*Bromus*	*sterilis*	*Poa*	*trivialis*	*Bombus*	*bohemicus*
31	*Calamagrostis*	*canescens*	*Polygonum*	*aviculare*	*Bombus*	*hypnorum*
32	*Calamagrostis*	*epigejos*	*Populus*	*tremula*	*Bombus*	*lapidarius*
33	*Calystegia*	*sepium*	*Potamogeton*	*acutifolius*	*Bombus*	*lucorum*
34	*Capsella*	*bursa-pastoris*	*Potamogeton*	*natans*	*Bombus*	*muscorum*
35	*Carduus*	*crispus*	*Potentilla*	*reptans*	*Bombus*	*pascuorum*
36	*Carduus*	*nutans*	*Prunus*	*avium*	*Bombus*	*pratorum*
37	*Carex*	*acuata*	*Prunus*	*padus*	*Bombus*	*ruderarius*
38	*Carex*	*elata*	*Prunus*	*serotina*	*Bombus*	*semenoviellus*
39	*Carex*	*pseudocyperus*	*Prunus*	*spinosa*	*Bombus*	*soroeensis*
40	*Carex*	*riparia*	*Quercus*	*robur*	*Bombus*	*sylvarum*
41	*Carex*	*versicaria*	*Quercus*	*rubra*	*Bombus*	*sylvestris*
42	*Carex*	*vulpina*	*Ranunculus*	*aquatilis*	*Bombus*	*terrestris*
43	*Ceratophyllum*	*submersum*	*Ranunculus*	*repens*	*Bombus*	*vestalis*
44	*Characeae*		*Ranunculus*	*sceleratus*	*Colletes*	*cunicularius*
45	*Chenopodium*	*album*	*Riccia*	*fluitans*	*Hylaeus*	*communis*
46	*Chenopodium*	*rubrum*	*Rorippa*	*amphibia*	*Hylaeus*	*confusus*
47	*Cirsium*	*arvensis*	*Rorippa*	*palustris*	*Halictus*	*maculatus*
48	*Cirsium*	*palustris*	*Rosa*	*sp.*	*Halictus*	*quadricinctus*
49	*Cirsum*	*vulgaris*	*Rubus*	*sp.*	*Halictus*	*rubicundus*
50	*Comarum*	*palustre*	*Rumex*	*crispus*	*Halictus*	*sexcinctus*
51	*Convolvulus*	*arvensis*	*Rumex*	*obtusifolius*	*Halictus*	*tumulorum*
52	*Corylus*	*avellana*	*Rumex*	*palustris*	*Lasioglossum*	*calceatum*
53	*Crataegus*	*sp.*	*Salix*	*alba*	*Lasioglossum*	*lativentre*
54	*Cyanus*	*segetum*	*Salix*	*cinerea*	*Lasioglossum*	*leucopus*
55	*Dactylis*	*glomerata*	*Salix*	*fragilis*	*Lasioglossum*	*leucozonium*
56	*Deschampsia*	*cespitosa*	*Salix*	*pentandra*	*Lasioglossum*	*lineare*
57	*Descurainia*	*sophia*	*Salix*	*purpurea*	*Lasioglossum*	*minutissimum*
58	*Dryopteris*	*carthusiana*	*Salix*	*triandra*	*Lasioglossum*	*morio*
59	*Dryopteris*	*dilatata*	*Salix*	*viminalis*	*Lasioglossum*	*nitidiusculum*
60	*Dryopteris*	*filix-mas*	*Sambucus*	*nigra*	*Lasioglossum*	*pallens*
61	*Eleocharis*	*vulgaris*	*Schoenoplectus*	*lacustris*	*Lasioglossum*	*parvulum*
62	*Elymus*	*repens*	*Scrophularia*	*nodosa*	*Lasioglossum*	*pauxillum*

63	*Epilobium*	*ciliatum*	*Scutellaria*	*galericulata*	*Lasioglossum*	*quadrinotatum*
64	*Epilobium*	*hirsutum*	*Silene*	*dioica*	*Lasioglossum*	*sexnotatum*
65	*Epilobium*	*sp.*	*Silene*	*latifolia*	*Lasioglossum*	*spec.*
66	*Epilobium*	*tetragonum*	*Sisymbrium*	*officinale*	*Lasioglossum*	*subfasciatum*
67	*Equisetum*	*arvense*	*Solanum*	*dulcamara*	*Lasioglossum*	*villosulum*
68	*Equisetum*	*fluviatile*	*Sonchus*	*asper*	*Lasioglossum*	*xanthopus*
69	*Euonymus*	*europaeus*	*Sonchus*	*palustris*	*Lasioglossum*	*zonulum*
70	*Fagus*	*sylvatica*	*Sorbus*	*aucuparia*	*Nomada*	*bifasciata*
71	*Fraxinus*	*excelsior*	*Sparganium*	*erectum*	*Nomada*	*flavoguttata*
72	*Galeopsis*	*tetrahit*	*Spirodela*	*polyrhiza*	*Nomada*	*goodeniana*
73	*Galium*	*aparine*	*Stachys*	*palustris*	*Nomada*	*marshamella*
74	*Galium*	*palustre*	*Stachys*	*sylvestris*	*Nomada*	*minuscula cf.*
75	*Geranium*	*pusillum*	*Stellaria*	*aquatica*	*Nomada*	*moeschleri*
76	*Geum*	*urbanum*	*Stellaria*	*graminea*	*Nomada*	*panzeri*
77	*Glechoma*	*hederacea*	*Stellaria*	*media*	*Nomada*	*ruficornis*
78	*Glyceria*	*fluitans*	*Stellaria*	*palustris*	*Osmia*	*bicornis*
79	*Glyceria*	*maxima*	*Symphoricarpos*	*albus*	*Osmia*	*leaiana*
80	*Gnaphalium*	*uliginosum*	*Symphytum*	*officinale*	*Osmia*	*mustelina*
81	*Heracleum*	*sphondylium*	*Taraxacum*	*ruderale*	*Osmia*	*uncinata*
82	*Holcus*	*lanatus*	*Tephroseris*	*palustris*	*Sphecodes*	*ephippius*
83	*Holcus*	*mollis*	*Thelypteris*	*palustris*	*Sphecodes*	*miniatus*
84	*Hottonia*	*palustris*	*Thlaspi*	*arvense*	*Sphecodes*	*monilicornis*
85	*Humulus*	*lupulus*	*Tilia*	*sp.*		
86	*Hypericum*	*perforatum*	*Trifolium*	*pratensis*		
87	*Impatiens*	*glandulifera*	*Trifolium*	*repens*		
88	*Impatiens*	*parviflora*	*Tripleurospermum*	*inodorum*		
89	*Iris*	*pseudacorus*	*Typha*	*angustifolia*		
90	*Juncus*	*bufonius*	*Typha*	*latifolia*		
91	*Juncus*	*effusus*	*Urtica*	*dioica*		
92	*Lamium*	*purpureum*	*Veronica*	*chamaedrys*		
93	*Lapsana*	*communis*	*Veronica*	*scutellata*		
94	*Larix*	*europaeus*	*Vicia*	*cracca*		
95	*Lathyrus*	*latifolius*	*Vicia*	*hirsuta*		
96	*Lathyrus*	*pratensis*	*Vicia*	*sativa ssp. nigra*		
97	*Lemna*	*gibba*	*Vicia*	*sp.*		
98	*Lemna*	*minor*	*Vicia*	*tetrasperma*		
99	*Lemna*	*trisulca*	*Vicia*	*villosa*		
100			*Viola*	*arvensis*		

Fig. B1. Comparison of Multilocus Linkage Disequilibrium from microsatellite and binary datasets.

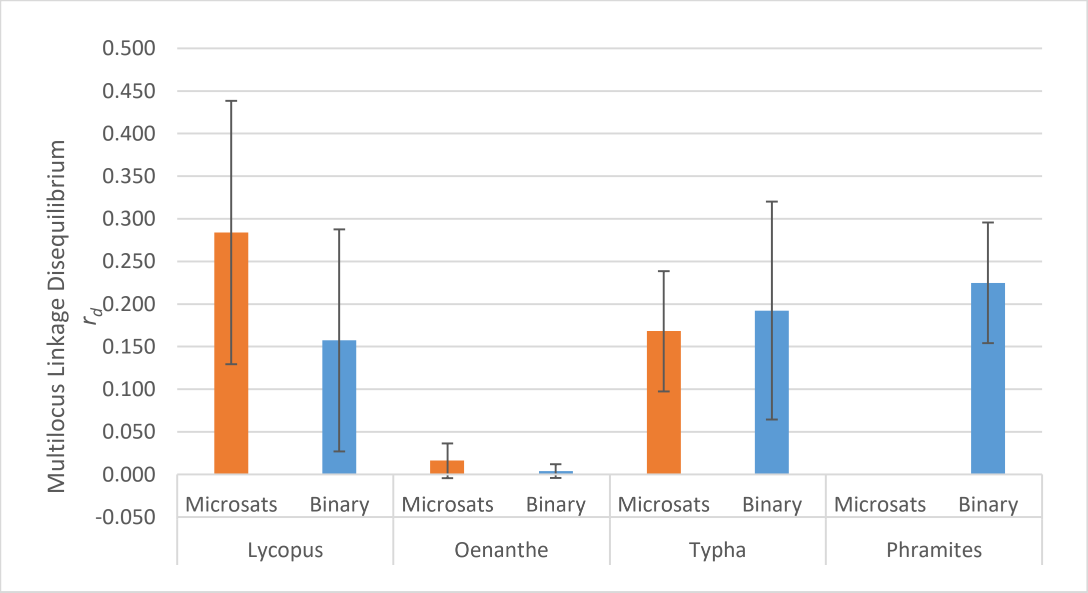


Table B2. Primer details used for microsatellites analyses.

Marker	Primer	Sequence	Repeat motif	Binding temperature	Reference	PCR conditions
Typha latifolia						
TL 146	F R	GGACTACGGTCCTTCTTTTT TGACAAGCACATTATTGACTTT	$(AT)_7$	62	Ciotir et al. 2013	Initial denaturation at 96 C for 160 s, followed by 30 cycles of 96 C for 20 s, 60 C for 50 s, and 72 C for 60 s with a final extension at 72 C for 10 min.
TL 209	F R	TGTCCTTTTTGTGTCACTTG TGCGTTATAGATGATATGGTTT	$(AG)_6$	62	Ciotir et al. 2013	
TL 247	F R	AGGCTAGCTAATAAGCCCTAA TCGAATACCCTTGAGAATGT	$(AAT)_4$	62	Ciotir et al. 2013	
TL 305	F R	CTTACCAGTTCCAAATTCCA AGCATGCTTAACAACCAAGT	$(CT)_6$	62	Ciotir et al. 2013	
TL 368	F R	ATTATTCCCTTGCAGACCA GAATTGAAGTCCTCCTATCAAA	$(GT)_8$	62	Ciotir et al. 2013	
TA3	F R	GAGTTGGGAAGAAGGGATTA TGGATACGGCAGTGTTA	$(AC)_{12}...(AG)_{13}$	60	Tsyusko-Omeltchenko et al. 2003	
TA7	F R	ATTCAACCCAAACTCTAACAA CACCCAAAGGACCACATT	$(AC)_9...(AG)_{17}$	60	Tsyusko-Omeltchenko et al. 2003	
TA8	F R	TCTTCGCTGAAAGTGACATAC ATTGGCTTCGTTGGATT	$(AC)_{11}$	60	Tsyusko-Omeltchenko et al. 2003	
T20	F R	FAM ATGCCTAGTGAGGATTC CACACTTATTTTCGAACAA	$(AG)_{10}$	60	Tsyusko-Omeltchenko et al. 2003	
Tmin01	F R	CTTCTTCTCGTGTCCACCG TGCAGTACGGCCTCATCG	(AG)	57	Csencsics et al. 2010	Initial denaturing at 94 C for 15 min, followed by 30 cycles of 94 C for 30 s, 57 C for 90 s, and 72 C for 1 min, followed by 8 cycles of 94 C for 30 s, 53 C for 90 s, and 72 C for 1 min with a final extension of 72 C for 30 min.
Lycopus europaeus						
L_10	F R	TCAAGGAAAATCAGCAAGATTC CCAATCTGTGGTATTCGAACTG	$(TC)_{11}$	57	Favre-Bac et al. 2014	Initial denaturation of 95 C for 15 min; 30 cycles of 94 C for 30 s, annealing temperature for 1 min 30 s, and 72 C for 1 min; and a final extension at 60 C for 30 min.
L_11	F R	CTCGAGAGCGAAGGCAAA CCTGAGAAGAGTTCATTGAGCA	$(CT)_{12}$	57	Favre-Bac et al. 2014	
L_15	F R	GATACTGGCGTAGAAGATCGAA TCACGTTTACTGCATGTGGTC	$(GA)_{12}$	57	Favre-Bac et al. 2014	
L_16	F R	GATTTTCTGCCGGCTTACAC CAAACTGTGTTGGAATGGCA	$(TC)_{13}$	57	Favre-Bac et al. 2014	
L_17	F R	GCCCTTCTTTTTGTGGTCG CGGAGCTTCCTCTCAACAAC	$(TC)_{13}$	57	Favre-Bac et al. 2014	
L_18	F R	CAGATCTGGGACACCGCT TCCAGCAAAACGTTACATGC	$(TG)_{13}$	57	Favre-Bac et al. 2014	
L_19	F R	TTCATATTGCTCGTGATTCATT GCATGTATTTTGGTTAGATATCAGG	$(GA)_{13}$	57	Favre-Bac et al. 2014	
L_23	F R	GATGCTCTCAAAGAGGTGGG GAGAAACCTAGACTCCACAACTGA	$(TCT)_{14}$	57	Favre-Bac et al. 2014	
L_33	F R	GATGATGGGAATAAGCCGTG TCATTTTCTTCGCAGCATGA	$(GA)_{16}$	57	Favre-Bac et al. 2014	
L_35	F	CTCGCTCTGCAGAAACACAA	$(AC)_{17}$	57	Favre-Bac et al. 2014	

	R	AAGACAGAGTTCTCGTGCCA				
L_38	F	TAGACATGCTTTGTTTGATTGATATT	$(CA)_{18}$	57	Favre-Bac et al. 2014	
	R	GACAGCAGCACCTGCAAAT				
L_40	F	GTATAGGAAAAGGGAAGGAAAAA	$(GA)_{19}$	57	Favre-Bac et al. 2014	
	R	CAAGTACACGGTGAGATTCTGC				
L_42	F	TACAAAAGGAGTCGCACCGT	$(AG)_{19}$	57	Favre-Bac et al. 2014	
	R	GGGAACAAGCTTTTGGCTTT				
L_45	F	ACCATTCTACAATGCAACCG	$(GA)_{19}$	57	Favre-Bac et al. 2014	
	R	ACAAAACACATCATGGCATATCA				
L_48	F	GGCACTAGTTCCACTTAATTGCC	$(CA)_{10}$	57	Favre-Bac et al. 2014	
	R	TGCAGAAATGGTAGGATAATGG				
Oenanthe aquatica						
O_01	F	AGTCGCAAGTTAAAGGGAAGC	$(AGC)_6$	57	Favre-Bac et al. 2014	Initial denaturation of 95 C for 15 min; 30 cycles of 94 C for 30 s, annealing temperature for 1 min 30 s, and 72 C for 1 min; and a final extension at 60 C for 30 min.
	R	GGAGCATACTCTTGGGAGAGG				
O_03	F	AATGTCATTCATCCCACCAC	$(TTG)_7$	57	Favre-Bac et al. 2014	
	R	TGGGTTCCATGCAAAATTATC				
O_10	F	ACATCGATCTGGGCTGGTAA	$(CA)_{11}$	57	Favre-Bac et al. 2014	
	R	ATTTAATTCAAGGCGTTGCG				
O_13	F	ACACAAGATTTATAATCTGGCAAA	$(AC)_{11}$	57	Favre-Bac et al. 2014	
	R	GCAATGACATAGTCCAAGCTG				
O_17	F	ATCTCAGTGTTATGTGCTGTGTAGA	$(TC)_{12}$	54	Favre-Bac et al. 2014	
	R	TCACCGGGCGTTGAATAATA				
O_18	F	TACACGAAAGCGACGGTGAT	$(AG)_{12}$	57	Favre-Bac et al. 2014	
	R	CATCAGGGTCCGATATGACA				
O_20	F	TTACCGTATTGTTAATTTTACCGGAG	$(TC)_{12}$	57	Favre-Bac et al. 2014	
	R	TTGCTCGAATTCCAACATAAA				
O_21	F	CACCCAACAAGAAACAGTACTATAAA	$(AG)_{12}$	57	Favre-Bac et al. 2014	
	R	TCAAAGCATTCTTGGCCTTC				
O_28	F	GCACTGTCCCGGTAAGTCTG	$(GT)_{13}$	54	Favre-Bac et al. 2014	
	R	AAGTTTGACTGATAAGGTTTCCA				
O_32	F	AGAAAACGGGGACGAAGAAG	$(TG)_{13}$	55	Favre-Bac et al. 2014	
	R	CACCAAGAAGCGACTCCACT				
O_37	F	TCGATAGCCACAAGAGCAAA	$(GA)_{15}$	57	Favre-Bac et al. 2014	
	R	TTACAATCATGGCTTCGTGA				
O_38	F	CAATCCAACACTCTCATTTTCC	$(AC)_{15}$	55	Favre-Bac et al. 2014	
	R	TCCTAAGCAAAGTCATCAATGC				
O_47	F	CCATCGATAGCATCCAGGTA	$(TG)_{18}$	57	Favre-Bac et al. 2014	
	R	AATAGTAATTAGGAATCTCACGCAC				
Phragmites australis						
PaGT4	F	TGCTCCCTGCCAGTTTCTTG	$(CA)_9$	56	Saltonstall 2003	Initial denaturation at 94 C for 12 min, followed by 35 iterations of 94 C for 30 s, 50–56 C for 30 s and 72 C for 4 s, and finally a 2-min extension at 72 C.
	R	TATCCACCCTTCGAAGGCAC				
PaGT8	F	TCTGAACATAATCCTGGTGG	$(CA)_8$	50	Saltonstall 2003	
	R	TCTGTGTGAAGCAGTTCTGC				
PaGT9	F	CCATGTGTTAATGTTGTCC	$(CA)_{10}$	46	Saltonstall 2003	
	R	ATTGAATCCACACGTTTCCG				
PaGT11	F	CAACTCCGTGAATGACATGC	$(CA)_8$	50	Saltonstall 2003	
	R	CAGTTTGTGCACTAATGGAC				

PaGT12	F R	CTTCCTAGGTCAGTATCATCC GTGGCAGCTGATTGATTTGG	$(CA)_9$	50	Saltonstall 2003
PaGT13	F R	CTCATGCATCACTTCACAGG ACACGGACCTAACATCAACC	$(CA)_9$	52	Saltonstall 2003
PaGT14	F R	GTTGCAGCAAGTATTTGG CAAGCATTCTAGTAGTAGC	$(CA)_7$	46	Saltonstall 2003
PaGT16	F R	ACCAATCAGTCAGACTAGCC GTTCTCATGTTGGAGAAGCC	$(CA)_{10}$	52	Saltonstall 2003
PaGT21	F R	GCTACTCAACAGGTATACGG ATTGAGGATTGAGGTGGTGG	$(CA)_5$ $(AT)_6$ $(CA)_6$	50	Saltonstall 2003
PaGT22	F R	TTGAGTGCCTGGTGTATTCG AAGCTTCTGTCATGGAACCG	$(AC)_8CTT$ $(GA)_5$	52	Saltonstall 2003

Table B3. Pairwise matrices of F_{ST} of *Oenanthe aquatica*.

	Boi_28	**Buc_1598**	**Buc_2565**	**Dam_910**	**Kra_805**	**Kra_807**	**Raa_1189**	**Rit_258**	**Rit_259**	**Rit_275**	**Wil_235**	**Wil_236**
Boi_28	----	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Buc_1598	0.265	----	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Buc_2565	0.301	0.141	----	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Dam_910	0.238	0.205	0.176	----	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Kra_805	0.225	0.145	0.135	0.161	----	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Kra_807	0.232	0.167	0.155	0.159	0.037	----	0.001	0.001	0.001	0.001	0.001	0.001
Raa_1189	0.256	0.115	0.144	0.168	0.085	0.100	----	0.001	0.001	0.001	0.001	0.001
Rit_258	0.203	0.145	0.123	0.118	0.040	0.040	0.093	----	0.001	0.001	0.001	0.001
Rit_259	0.205	0.150	0.118	0.118	0.077	0.092	0.102	0.048	----	0.001	0.001	0.001
Rit_275	0.270	0.210	0.212	0.244	0.075	0.100	0.151	0.076	0.113	----	0.001	0.001
Wil_235	0.235	0.120	0.116	0.178	0.103	0.116	0.115	0.090	0.103	0.158	----	0.003
Wil_236	0.241	0.169	0.179	0.188	0.108	0.112	0.114	0.091	0.117	0.157	0.030	----

F_{ST}Values below diagonal. Probability, P(rand >= data) based on 9999 permutations is shown above diagonal.

Table B4. Pairwise matrices of F_{ST} of *Lycopus europaeus*.

	Boi_28	**Buc_1598**	**Buc_1604**	**Buc_2565**	**Dam_907**	**Dam_911**	**Fal_190**	**Kra_805**
Boi_28	----	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Buc_1598	0.218	----	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Buc_1604	0.294	0.290	----	<0.001	<0.001	<0.001	<0.001	<0.001
Buc_2565	0.255	0.209	0.297	----	<0.001	<0.001	<0.001	<0.001
Dam_907	0.366	0.361	0.477	0.280	----	<0.001	<0.001	<0.001
Dam_911	0.276	0.321	0.401	0.329	0.411	----	<0.001	<0.001
Fal_190	0.380	0.409	0.471	0.420	0.512	0.426	----	<0.001
Kra_805	0.364	0.358	0.476	0.396	0.504	0.405	0.474	----

Fst Values below diagonal. Probability, P(rand >= data) based on 9999 permutations is shown above diagonal.

Table B5. Pairwise matrices of F_{ST} of *Typha latifolia*.

	Boi_28	**Buc_1604**	**Buc_1598**	**Dam_907**	**Dam_911**	**Kra_805**	**Raa_2484**	**Rit_275**
Boi_28	----	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Buc_1604	0.239	----	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Buc_1598	0.376	0.310	----	<0.001	<0.001	<0.001	<0.001	<0.001
Dam_907	0.458	0.419	0.206	----	<0.001	<0.001	<0.001	<0.001
Dam_911	0.306	0.117	0.352	0.450	----	<0.001	<0.001	<0.001
Kra_805	0.441	0.505	0.478	0.677	0.523	----	<0.001	<0.001
Raa_2484	0.427	0.303	0.419	0.527	0.407	0.405	----	<0.001
Rit_275	0.410	0.290	0.407	0.499	0.323	0.582	0.361	----

Fst Values below diagonal. Probability, P(rand >= data) based on 9999 permutations is shown above diagonal.

Table B6. Pairwise matrices of F_{ST} of *Phragmites australis*.

	Buc_1604	**Dam_892**	**Fal_183**	**Fal_190**	**Kra_805**	**Raa_607**	**Raa_2484**	**Rit_259**
Buc_1604	----	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Dam_892	0.077	----	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Fal_183	0.104	0.132	----	<0.001	<0.001	<0.001	<0.001	<0.001
Fal_190	0.092	0.146	0.109	----	<0.001	<0.001	<0.001	<0.001
Kra_805	0.039	0.100	0.084	0.083	----	<0.001	<0.001	<0.001
Raa_607	0.038	0.095	0.112	0.093	0.044	----	<0.001	<0.001
Raa_2484	0.082	0.143	0.137	0.117	0.083	0.077	----	<0.001
Rit_259	0.114	0.157	0.145	0.110	0.098	0.110	0.131	----

Table B7. Weighted matrix for the 20 selected kettle holes based on speed and direction of wind.

	Boi_28	**Buc_1598**	**Buc_1604**	**Buc_2565**	**Dam_892**	**Dam_907**	**Dam_910**	**Dam_911**	**Fal_183**	**Fal_190**	**Kra_805**	**Kra_807**	**Raa_1189**	**Raa_2484**	**Raa_607**	**Rit_258**	**Rit_259**	**Rit_275**	**Wil_235**	**Wil_236**
Boi_28	0.000	1.668	1.535	1.668	2.603	2.159	2.159	2.159	3.742	3.524	2.603	2.603	3.299	3.299	3.742	3.299	3.299	3.299	3.549	3.549
Buc_1598	1.668	0.000	1.657	1.668	2.159	1.657	1.657	1.657	3.742	3.742	2.159	2.159	2.603	2.603	3.299	3.299	3.299	3.299	3.549	3.524
Buc_1604	1.535	1.657	0.000	3.123	2.159	1.657	1.657	1.657	3.742	3.742	2.159	2.159	2.603	2.603	3.299	3.299	3.299	3.299	3.524	3.524
Buc_2565	1.668	1.668	3.123	0.000	2.159	1.657	1.657	1.657	3.742	3.742	2.159	2.159	2.159	2.603	2.603	3.299	2.603	2.603	3.524	3.524
Dam_892	2.603	2.159	2.159	2.159	0.000	3.549	3.123	2.658	1.668	1.535	1.141	1.535	1.657	1.242	1.242	1.668	1.668	1.668	1.141	1.141
Dam_907	2.159	1.657	1.657	1.657	3.549	0.000	3.299	1.657	1.668	1.535	1.668	2.464	1.242	1.242	0.989	1.668	2.464	2.464	1.350	1.350
Dam_910	2.159	1.657	1.657	1.657	3.123	3.299	0.000	1.350	1.668	1.535	1.535	1.668	1.242	1.242	0.989	1.668	1.668	2.464	1.141	1.350
Dam_911	2.159	1.657	1.657	1.657	2.658	1.657	1.350	0.000	1.668	1.535	1.535	1.668	1.242	1.242	0.989	1.668	1.668	2.464	1.350	1.350
Fal_183	3.742	3.742	3.742	3.742	1.668	1.668	1.668	1.668	0.000	3.742	1.668	1.668	3.549	3.524	3.524	1.668	1.668	1.535	1.242	1.242
Fal_190	3.524	3.742	3.742	3.742	1.535	1.535	1.535	1.535	3.742	0.000	1.535	1.535	3.549	3.549	3.524	1.141	1.141	1.141	0.989	0.989
Kra_805	2.603	2.159	2.159	2.159	1.141	1.668	1.535	1.535	1.668	1.535	0.000	2.603	2.159	1.657	1.657	2.464	2.464	2.658	1.141	1.141
Kra_807	2.603	2.159	2.159	2.159	1.535	2.464	1.668	1.668	1.668	1.535	2.603	0.000	2.159	2.159	1.657	1.668	2.464	2.464	1.141	1.141
Raa_1189	3.299	2.603	2.603	2.159	1.657	1.242	1.242	1.242	3.549	3.549	2.159	2.159	0.000	2.464	1.668	3.742	3.742	3.742	2.464	2.464
Raa_2484	3.299	2.603	2.603	2.603	1.242	1.242	1.242	1.242	3.524	3.549	1.657	2.159	2.464	0.000	1.535	3.299	3.299	3.299	2.464	2.464
Raa_607	3.742	3.299	3.299	2.603	1.242	0.989	0.989	0.989	3.524	3.524	1.657	1.657	1.668	1.535	0.000	3.299	3.299	3.299	2.464	2.464
Rit_258	3.299	3.299	3.299	3.299	1.668	1.668	1.668	1.668	1.668	1.141	2.464	1.668	3.742	3.299	3.299	0.000	1.078	1.657	1.078	1.078
Rit_259	3.299	3.299	3.299	2.603	1.668	2.464	1.668	1.668	1.668	1.141	2.464	2.464	3.742	3.299	3.299	1.078	0.000	2.159	1.078	1.078
Rit_275	3.299	3.299	3.299	2.603	1.668	2.464	2.464	2.464	1.535	1.141	2.658	2.464	3.742	3.299	3.299	1.657	2.159	0.000	1.078	1.078
Wil_235	3.549	3.549	3.524	3.524	1.141	1.350	1.141	1.350	1.242	0.989	1.141	1.141	2.464	2.464	2.464	1.078	1.078	1.078	0.000	1.078
Wil_236	3.549	3.524	3.524	3.524	1.141	1.350	1.350	1.350	1.242	0.989	1.141	1.141	2.464	2.464	2.464	1.078	1.078	1.078	1.078	0.000

Table B8. Similarity matrix based on Jaccard-Index of presence-absence data of bee species.

	Boi_28	**Buc_1598**	**Buc_1604**	**Buc_2565**	**Dam_892**	**Dam_907**	**Dam_910**	**Dam_911**	**Fal_183**	**Fal_190**	**Kra_805**	**Kra_807**	**Raa_1189**	**Raa_2484**	**Raa_607**	**Rit_258**	**Rit_259**	**Rit_275**	**Wil_235**	**Wil_236**
Boi_28	0.000	0.880	0.909	0.643	0.821	0.750	0.706	0.833	0.714	0.875	0.733	0.643	0.706	0.846	0.917	0.714	0.643	0.647	0.786	0.692
Buc_1598	0.880	0.000	0.800	0.852	0.816	0.667	0.786	0.731	0.742	0.846	0.808	0.808	0.786	0.920	0.810	0.750	0.852	0.750	0.840	0.792
Buc_1604	0.909	0.800	0.000	0.846	0.885	0.769	0.875	0.867	0.850	0.727	0.846	0.846	0.800	0.900	0.200	0.727	0.750	0.813	0.700	0.818
Buc_2565	0.643	0.852	0.846	0.000	0.759	0.789	0.684	0.737	0.696	0.688	0.778	0.625	0.684	0.875	0.857	0.765	0.842	0.632	0.824	0.750
Dam_892	0.821	0.816	0.885	0.759	0.000	0.767	0.700	0.914	0.581	0.833	0.667	0.759	0.742	0.897	0.889	0.793	0.800	0.710	0.786	0.741
Dam_907	0.750	0.667	0.769	0.789	0.767	0.000	0.632	0.750	0.652	0.842	0.850	0.722	0.762	0.882	0.786	0.778	0.789	0.650	0.833	0.688
Dam_910	0.706	0.786	0.875	0.684	0.700	0.632	0.000	0.714	0.429	0.800	0.810	0.529	0.667	0.833	0.882	0.667	0.913	0.474	0.647	0.563
Dam_911	0.833	0.731	0.867	0.737	0.914	0.750	0.714	0.000	0.720	0.850	0.857	0.667	0.826	0.824	0.875	0.647	0.857	0.727	0.900	0.778
Fal_183	0.714	0.742	0.850	0.696	0.581	0.652	0.429	0.720	0.000	0.792	0.636	0.636	0.680	0.818	0.857	0.619	0.800	0.455	0.667	0.667
Fal_190	0.875	0.846	0.727	0.688	0.833	0.842	0.800	0.850	0.792	0.000	0.895	0.833	0.800	0.938	0.750	0.750	0.895	0.750	0.733	0.882
Kra_805	0.733	0.808	0.846	0.778	0.667	0.850	0.810	0.857	0.636	0.895	0.000	0.842	0.684	0.875	0.857	0.833	0.706	0.762	0.824	0.667
Kra_807	0.643	0.808	0.846	0.625	0.759	0.722	0.529	0.667	0.636	0.833	0.842	0.000	0.750	0.800	0.857	0.688	0.900	0.471	0.750	0.462
Raa_1189	0.706	0.786	0.800	0.684	0.742	0.762	0.667	0.826	0.680	0.800	0.684	0.750	0.000	0.950	0.813	0.737	0.750	0.619	0.789	0.722
Raa_2484	0.846	0.920	0.900	0.875	0.897	0.882	0.833	0.824	0.818	0.938	0.875	0.800	0.950	0.000	0.909	0.786	0.941	0.842	0.933	0.769
Raa_607	0.917	0.810	0.200	0.857	0.889	0.786	0.882	0.875	0.857	0.750	0.857	0.857	0.813	0.909	0.000	0.750	0.769	0.824	0.727	0.833
Rit_258	0.714	0.750	0.727	0.765	0.793	0.778	0.667	0.647	0.619	0.750	0.833	0.688	0.737	0.786	0.750	0.000	0.833	0.529	0.733	0.733
Rit_259	0.643	0.852	0.750	0.842	0.800	0.789	0.913	0.857	0.800	0.895	0.706	0.900	0.750	0.941	0.769	0.833	0.000	0.818	0.824	0.824
Rit_275	0.647	0.750	0.813	0.632	0.710	0.650	0.474	0.727	0.455	0.750	0.762	0.471	0.619	0.842	0.824	0.529	0.818	0.000	0.667	0.500
Wil_235	0.786	0.840	0.700	0.824	0.786	0.833	0.647	0.900	0.667	0.733	0.824	0.750	0.789	0.933	0.727	0.733	0.824	0.667	0.000	0.714
Wil_236	0.692	0.792	0.818	0.750	0.741	0.688	0.563	0.778	0.667	0.882	0.667	0.462	0.722	0.769	0.833	0.733	0.824	0.500	0.714	0.000

Fig. B2. STRUCTURE results showing barplots and most probable K groups in *Oenanthe aquatica*.

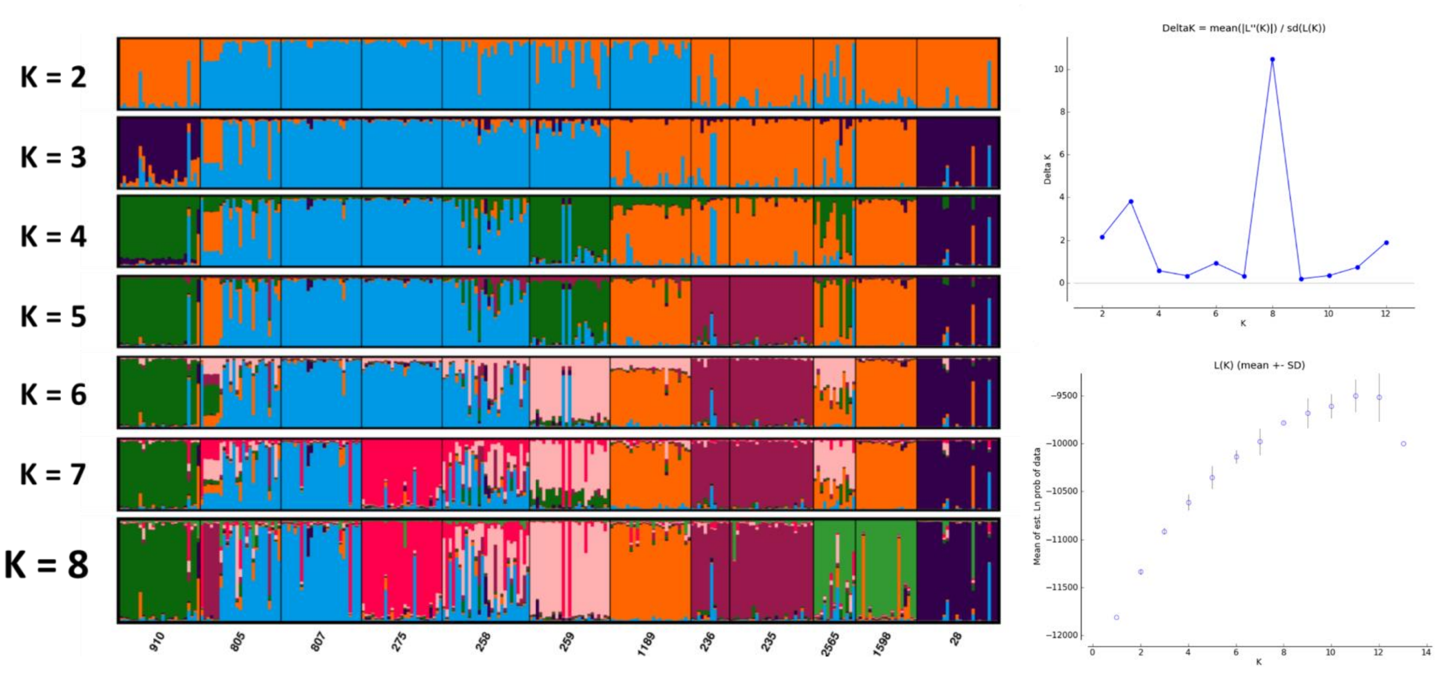


Fig. B3. STRUCTURE results showing barplots and most probable K groups in *Lycopus europaeus*.

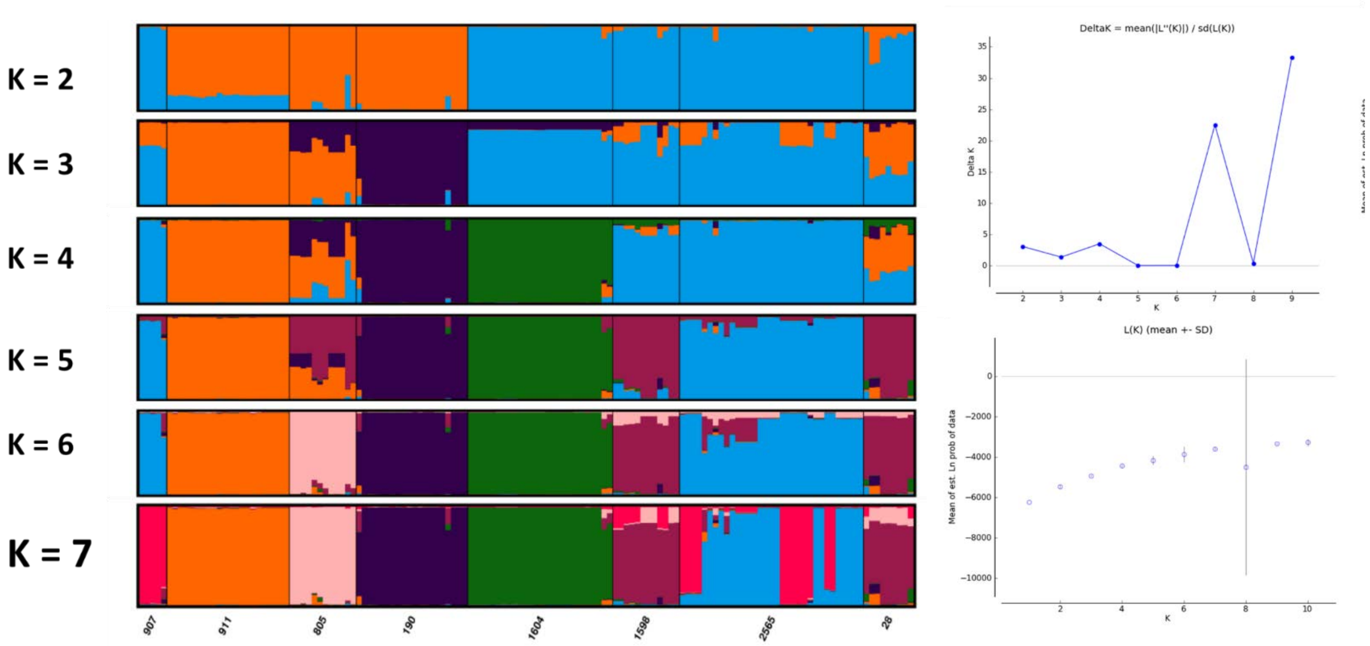


Fig. B4. STRUCTURE results showing barplots and most probable K groups in *Typha latifolia*.

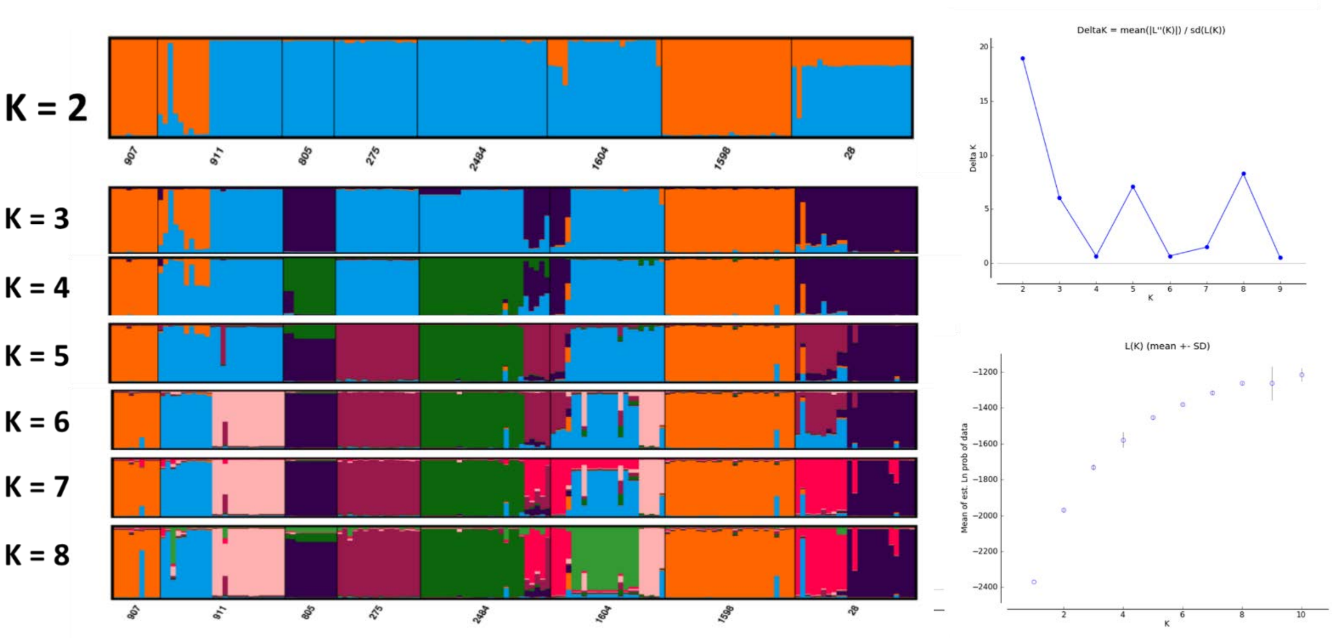


Fig. B5. STRUCTURE results showing barplots and most probable K groups in *Phragmites australis*.

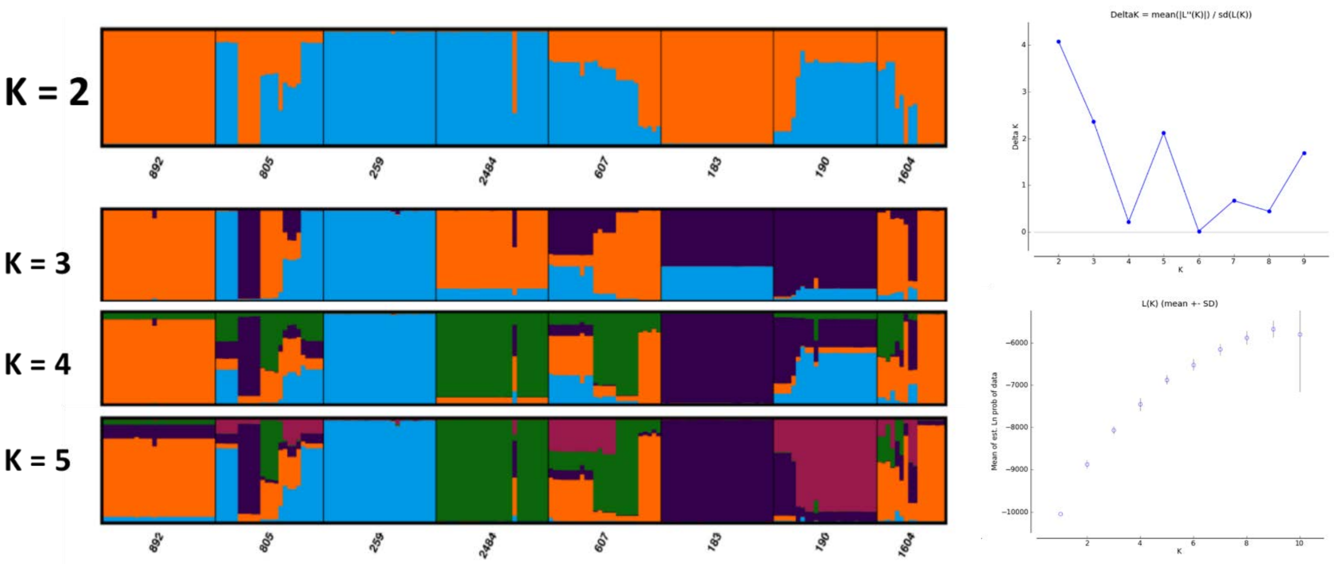


Fig. B6. Linear models representing the relationship between observed heterozygosity (H_O) with the landscape biotic and abiotic factors: Patch size (A), isolation degree (B), plant (C) and bee richness (D).

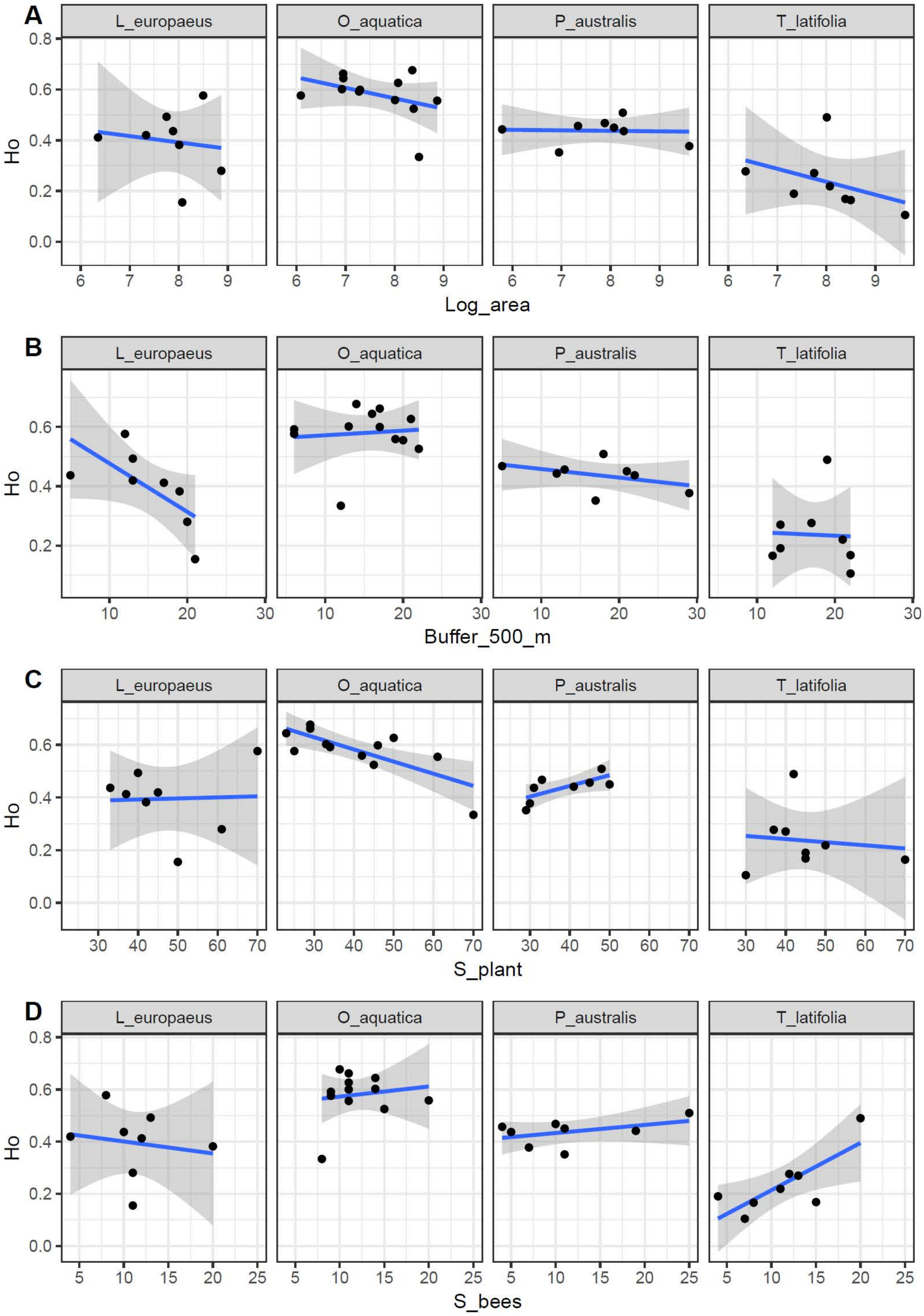


Fig. B7. Linear models representing the relationship between expected heterozygosity (H_E) with the landscape biotic and abiotic factors: Patch size (A), isolation degree (B), plant (C) and bee richness (D).

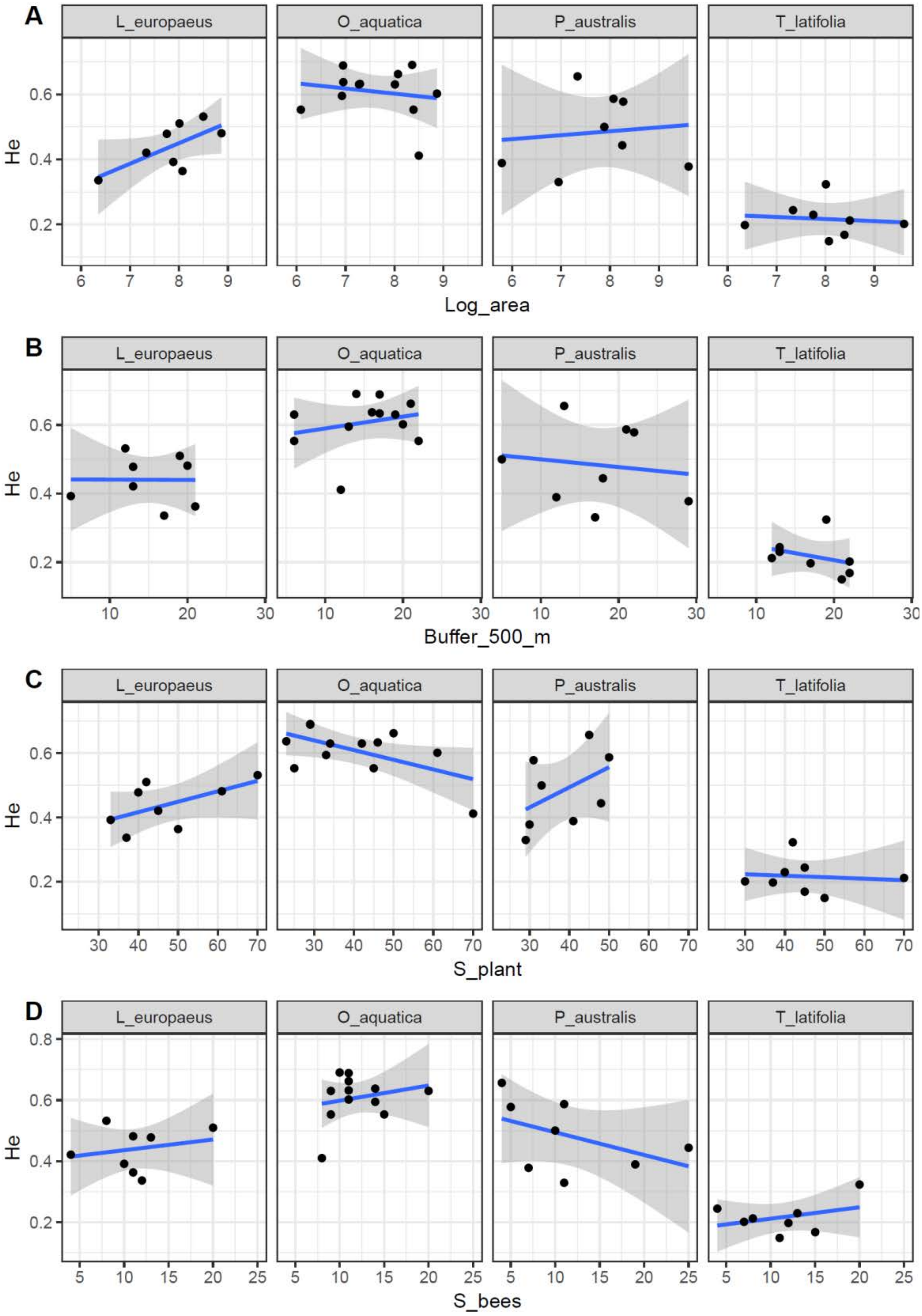
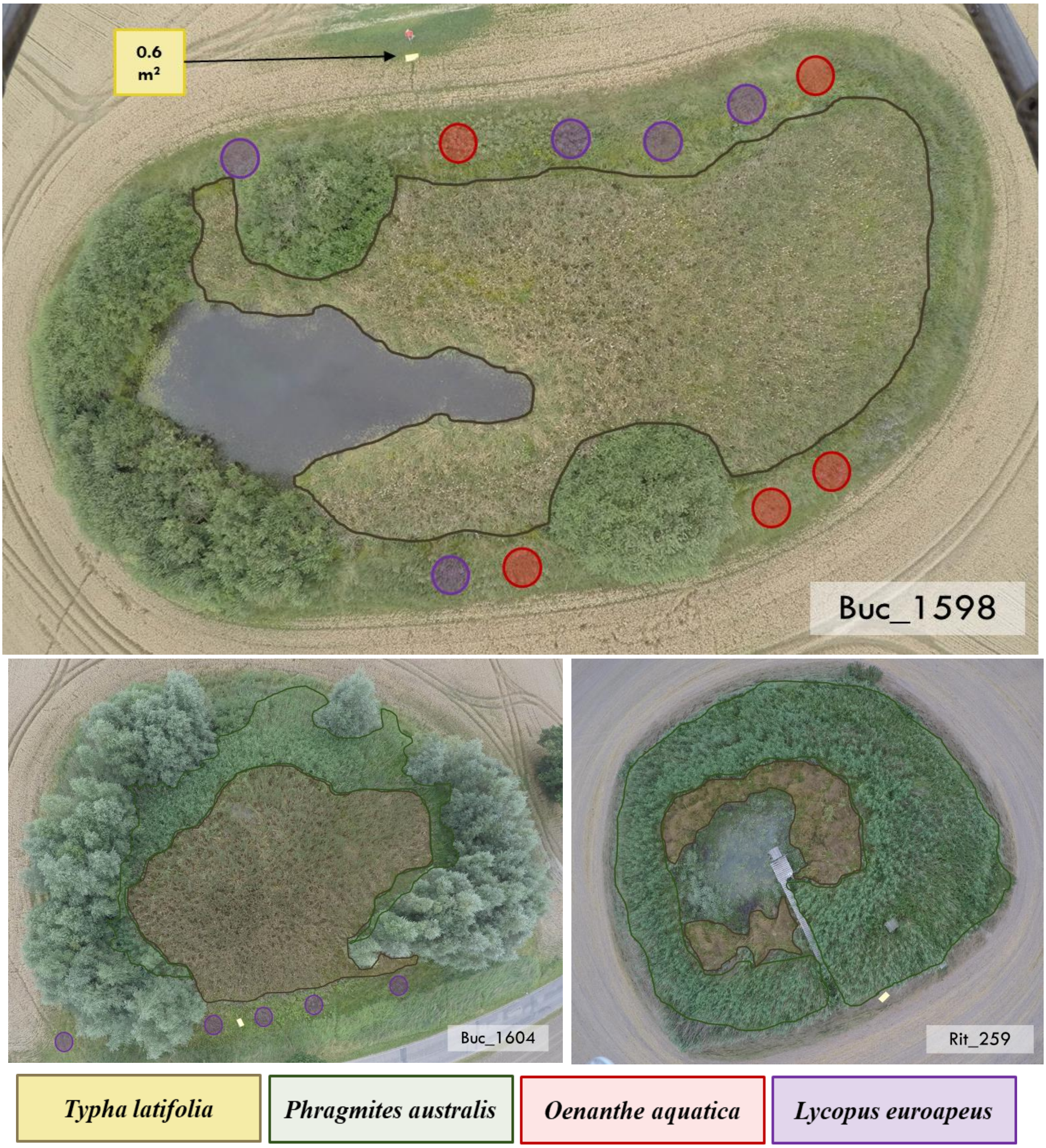


Fig. B8. Aerial photograph of one kettle hole showing the population size of the species. Examples of 3 kettle holes and the occurrence of the studied species. Circles represent an approximation of the location of *Lycopus europaeus* and *Oenanthe aquatica* since the small populations of these species were not visible from the aerial photographs taken by the drone.



APPENDIX C

Supplementary Material to Chapter 4

List of tables

Table C1: List of bee species and their functional traits found in the kettle holes.

Table C2: Estimate of % plant cover based on Londo scale (Londo 1976).

Table C3: Characteristics of the selected kettle holes and bee diversity characterized by species richness (S) and Shannon Index (H).

Table C4: Details for the model selection based to test the effect of number of kettle holes occurring at different radii (degree of isolation) on bee species richness and abundance.

Table C5: Definitions of bees functional traits taken from Kratschmer et al. (2019).

List of figures

Fig. C1: Color traps used to sample bees in the kettle holes.

Fig. C2: Rarefaction accumulation curve of species based on 9999 permutations.

Fig. C3: Wild-bee diversity according type of cropland.

Fig. C4: Proportion of small, medium and large species according functional traits.

Fig. C5: Relationship between bee species richness according to body sizes with degree of isolation.

Table C1. List of bee species and their functional traits found in the kettle holes.

	Genus	Species	Code	# ind	Body length	Sociality	Lecty	Nesting type
1	***Andrena***	*anthrisci*	Aant	1	Small	Solitary	Poly	Belowground
	Andrena	*anthrisci cf.*	Aant_cf	1				
2	***Andrena***	*bicolor*	Abic	1	Small	Solitary	Poly	Belowground
3	***Andrena***	*carantonica**	Acar	5	Medium	Eusocial	Poly	Belowground
4	***Andrena***	*chrysosceles*	Achr	6	Small	Solitary	Poly	Belowground
5	***Andrena***	*cineraria*	Acin	22	Medium	Solitary	Poly	Belowground
6	***Andrena***	*dorsata*	Ador	1	Small	Solitary	Poly	Belowground
7	***Andrena***	*flavipes**	Afla	85	Medium	Solitary	Poly	Belowground
8	***Andrena***	*fucata*	Afuc	2	Medium	Solitary	Poly	Belowground
9	***Andrena***	*fulva**	Aful	8	Medium	Solitary	Poly	Belowground
10	***Andrena***	*gravida**	Agra	62	Medium	Solitary	Poly	Belowground
11	***Andrena***	*haemorrhoa**	Ahae	237	Medium	Solitary	Poly	Belowground
12	***Andrena***	*helvola*	Ahel	14	Medium	Solitary	Poly	Belowground
	Andrena	*helvola cf.*	Ahel_cf	1				
13	***Andrena***	*labiata*	Alab	1	Small	Solitary	Poly	Belowground
14	***Apis***	*mellifera*	Amel	39				
15	***Andrena***	*minutula**	Amina	5	Small	Solitary	Poly	Belowground
16	***Andrena***	*minutuloides*	Amin	2	Small	Solitary	Poly	Belowground
17	***Andrena***	*nigroaenea**	Anig	189	Medium	Solitary	Poly	Belowground
18	***Andrena***	*nitida**	Anit	98	Medium	Solitary	Poly	Belowground
19	***Andrena***	*pilipes*	Apil	1	Medium	Solitary	Poly	Belowground
20	***Andrena***	*praecox*	Apra	3	Medium	Solitary	Oligo	Belowground
21	***Andrena***	*strohmella*	Astr	1	Small	Solitary	Poly	Belowground
22	***Andrena***	*subopaca*	Asub	11	Small	Solitary	Poly	Belowground
23	***Andrena***	*suerinensis*	Asue	1	Medium	Solitary	Oligo	Belowground
24	***Andrena***	*tibialis**	Atib	3	Medium	Solitary	Poly	Belowground
25	***Andrena***	*vaga*	Avag	6	Medium	Solitary	Oligo	Belowground
26	***Andrena***	*ventralis*	Aven	2	Small	Solitary	Oligo	Belowground
27	***Andrena***	*wilkella*	Awil	1	Medium	Solitary	Oligo	Belowground
28	***Bombus***	*bohemicus*	Bboh	3	Large	Parasitic	Poly	Parasitic
29	***Bombus***	*lapidaries**	Blap	1	Large	Eusocial	Poly	Above and belowground
30	***Bombus***	*muscorum*	Bmus	1	Large	Eusocial	Poly	Aboveground
31	***Bombus***	*pascuorum**	Bpas	4	Large	Eusocial	Poly	Aboveground
32	***Bombus***	*pratorum**	Bpra	5	Large	Eusocial	Poly	Belowground
33	***Bombus***	*ruderarius*	Brud	1	Large	Eusocial	Poly	Aboveground
34	***Bombus***	*semenoviellus*	Bsem	1	Large	Eusocial	Poly	Belowground
35	***Bombus***	*soroeensis*	Bsor	1	Large	Eusocial	Poly	Belowground
36	***Bombus***	*sylvarum*	Bsylum	6	Large	Parasitic	Poly	Parasitic
37	***Bombus***	*terrestris**	Bter	8	Large	Eusocial	Poly	Belowground

38	***Bombus***	*vestalis*	Bves	1	Large	Parasitic	Poly	Parasitic
39	***Colletes***	*cunicularius**	Ccun	1	Medium	Solitary	Poly	Belowground
40	***Halictus***	*maculatus*	Hmac	11	Small	Eusocial	Poly	Belowground
41	***Halictus***	*quadricinctus*	Hqua	53	Large	Eusocial	Poly	Belowground
42	***Halictus***	*rubicundus*	Hrub	1	Medium	Eusocial	Poly	Belowground
43	***Halictus***	*sexcinctus*	Hsex	1	Medium	Solitary	Poly	Belowground
44	***Halictus***	*tumulorum*	Htum	35	Small	Eusocial	Poly	Belowground
45	***Lasioglossum***	*calceatum*	Lcal	38	Small	Eusocial	Poly	Belowground
46	***Lasioglossum***	*lativentre*	Llat	2	Small	Solitary	Poly	Belowground
47	***Lasioglossum***	*leucopus*	Lleus	3	Small	Solitary	Poly	Belowground
48	***Lasioglossum***	*leucozonium*	Lleu	1	Small	Solitary	Poly	Belowground
49	***Lasioglossum***	*lineare*	Llin	1	Small	Eusocial	Poly	Belowground
50	***Lasioglossum***	*minutissimum*	Lmin	5	Small	Solitary	Poly	Belowground
51	***Lasioglossum***	*morio*	Lmor	11	Small	Eusocial	Poly	Belowground
52	***Lasioglossum***	*nitidiusculum**	Lnit	2	Small	Solitary	Poly	Belowground
53	***Lasioglossum***	*pallens*	Lpal	1	Small	Solitary	Poly	Belowground
54	***Lasioglossum***	*parvulum*	Lpar	9	Small	Solitary	Poly	Belowground
55	***Lasioglossum***	*pauxillum*	Lpau	156	Small	Eusocial	Poly	Belowground
56	***Lasioglossum***	*quadrinotatum**	Lqua	67	Small	Solitary	Poly	Belowground
57	***Lasioglossum***	*sexnotatum*	Lsex	3	Medium	Solitary	Poly	Belowground
58	***Lasioglossum***	*spec.*	Lspe	4				
59	***Lasioglossum***	*subfasciatum*	Lsub	1	Small	Solitary	Poly	Belowground
60	***Lasioglossum***	*villosulum**	Lvil	3	Small	Solitary	Poly	Belowground
61	***Lasioglossum***	*xanthopus**	Lxan	87	Medium	Solitary	Poly	Belowground
62	***Lasioglossum***	*zonulum*	Lzon	4	Small	Solitary	Poly	Belowground
63	***Nomada***	*bifasciata*	Nbif	4	Medium	Parasitic	Poly	Parasitic
64	***Nomada***	*flavoguttata*	Nfla	3	Small	Parasitic	Poly	Parasitic
65	***Nomada***	*goodeniana*	Ngoo	1	Medium	Parasitic	Poly	Parasitic
66	***Nomada***	*marshamella*	Nmar	1	Medium	Parasitic	Poly	Parasitic
67	***Nomada***	*minuscula cf.*	Nmin_cf	1				
68	***Nomada***	*moeschleri*	Nmoe	2	Small	Parasitic	Poly	Parasitic
69	***Nomada***	*panzeri*	Npan	3	Small	Parasitic	Poly	Parasitic
70	***Nomada***	*ruficornis*	Nruf	7	Small	Parasitic	Poly	Parasitic
71	***Osmia***	*bicornis*	Obic	1	Small	Solitary	Poly	Aboveground
72	***Osmia***	*leaiana*	Olea	1	Small	Solitary	Oligo	Aboveground
73	***Osmia***	*mustelina*	Omus	3	Medium	Solitary	Poly	Aboveground
74	***Osmia***	*uncinata*	Ounc	1	Small	Solitary	Poly	Aboveground
75	***Sphecodes***	*ephippius*	Seph	5	Small	Parasitic	Poly	Parasitic
76	***Sphecodes***	*miniatus*	Smin	2	Small	Parasitic	Poly	Parasitic
77	***Sphecodes***	*monilicornis*	Smon	3	Small	Parasitic	Poly	Parasitic

**These species were observed pollinating canola fields.*

Table C2. Estimate of % plant cover based on Londo scale (Londo 1976).

Coverage	Londo scale	Coverage	Londo scale
<1	0.1	35 – <45	40
1	1	45 – <55	50
>1 – <3	2	55 – <65	60
3 – <5	4	65 – <75	70
5 – <10	7	75 – <85	80
10 – <15	12	85 – <95	90
15 – <25	20	95 – 100	97.5
25 – <35	30		

Table C3. Characteristics of the selected kettle holes and bee diversity characterized by species richness (S) and Shannon Index (H). Altitude, area and perimeter are measured in meters. Hydro: Hydrogemorphological types of kettle holes S=Storage, SO=Shore Overflow, NPS=Non-Permanent Shore. "Veg" refers to the different types of dominant vegetation occurring all over the pond (Full) or in the edges (E) FR=Full with reed, FW=Full with wood, ER=Edge with reed, EW=Edge with wood, P= Puddle (open) type. Land use refers to the type of land surrounding the kettle hole, C=Cereal, M=Maize, G=Grassland, R=Rapeseed. Percent plant cover estimated based on a modified Londo scale (Table C2); some values are higher than 100 because plant cover was estimated in two different layers (herbs and trees). This estimation was not possible for all kettle holes (N=28). Flower cover was visually estimated in the field during the sampling of bee specimens. Species richness (S) and Shannon-Index (H) for diversity per kettle hole is shown for both bees and plants.

	Pond ID	Region	Longitude	Latitude	Altitude (m)	Area (m^2)	Perimeter (m)	Hydro	Veg	Shade %	Land use	Plant cover	Flower cover	S bees	H bees	S plants	H plants
1	**Boi_28**	Boisterfelde	13.529	53.318	101	4900	282.1	S	FR	25	C	NA	Low	8	2.0	NA	NA
2	**Boi_40**	Boisterfelde	13.537	53.317	97	3220	206.7	S	FW	100	M	110.7	Medium	15	2.4	31	0.7
3	**Buc_1338**	Buchenhain	13.557	53.310	91	1187	130.2	NPS	P	0	C	47.9	None	6	1.8	15	1.1
4	**Buc_1590**	Buchenhain	13.542	53.303	96	1117	132.2	S	FW	90	M	165.1	Medium	10	2.0	43	2.0
5	**Buc_1598**	Buchenhain	13.553	53.309	92	3009	222.4	S	EW	10	C	90.4	Low	20	2.5	42	1.3
6	**Buc_1599**	Buchenhain	13.552	53.309	92	733	105.6	SO	FR	0	C	99.6	None	11	2.2	13	0.5
7	**Buc_1604**	Buchenhain	13.551	53.306	89	1535	146.9	SO	FR	30	C	NA	None	4	1.3	NA	NA
8	**Buc_2565**	Buchenhain	13.559	53.306	89	7103	326.0	S	EW	80	C	NA	None	11	2.2	NA	NA

9	**Dam_892**	Damerow	13.651	53.406	90	3835	230.1	SO	ER	10	C	78.5	Low	25	2.5	30	1.0
10	**Dam_893**	Damerow	13.653	53.408	90	1910	178.3	NPS	P	0	C	36.9	Medium	5	1.6	12	1.2
11	**Dam_907**	Damerow	13.638	53.406	94	574	89.3	SO	ER	0	G	90	Low	12	2.1	33	2.2
12	**Dam_908**	Damerow	13.640	53.405	94	449	81.0	SO	FR	0	G	96.1	None	3	0.7	25	0.4
13	**Dam_910**	Damerow	13.642	53.407	95	1021	121.5	SO	ER	0	G	NA	None	14	2.4	NA	NA
14	**Dam_911**	Damerow	13.640	53.409	95	2330	192.4	SO	EW	0	G	NA	Very_low	13	2.4	NA	NA
15	**Fal_149**	Falkenhagen	13.758	53.350	63	5989	293.8	S	FW	100	C	NA	Very_low	6	1.5	NA	NA
16	**Fal_183**	Falkenhagen	13.743	53.367	63	327	69.3	S	EW	20	C	117.5	Medium	19	2.5	22	1.2
17	**Fal_187**	Falkenhagen	13.743	53.364	60	850	114.5	S	ER	0	C	107.1	Low	18	2.0	21	1.4
18	**Fal_190**	Falkenhagen	13.727	53.362	72	2653	197.9	S	FR	0	C	112.6	Low	10	1.8	33	1.4
19	**Kra_312**	Kraatz	13.686	53.387	79	7239	333.8	SO	ER	0	R	97.6	Low	9	1.8	26	2.1
20	**Kra_805**	Kraatz	13.662	53.395	87	3201	262.9	SO	EW	20	C	NA	Medium	11	1.8	NA	NA
21	**Kra_807**	Kraatz	13.666	53.397	87	1471	143.8	S	ER	5	C	118.1	Medium	11	2.0	41	1.9
22	**Kra_808**	Kraatz	13.668	53.397	88	3229	228.3	S	EW	25	C	NA	Very_low	10	1.8	NA	NA
23	**Par_1228**	Parmen	13.599	53.364	105	1988	167.7	S	EW	80	M	73.8	Low	15	2.3	42	2.2
24	**Par_1229**	Parmen	13.598	53.364	106	693	101.1	S	FW	100	M	110.8	Medium	14	2.5	26	0.8
25	**Raa_1189**	Raakow	13.618	53.354	88	1050	131.6	S	ER	0	M	113.8	Low	14	2.4	28	1.3
26	**Raa_2489**	Raakow	13.620	53.356	81	2448	183.6	SO	FR	0	M	104.7	None	11	1.9	14	0.9
27	**Raa_606**	Raakow	13.630	53.347	84	898	135.4	SO	FR	25	C	144.9	Medium	9	2.1	18	1.9
28	**Raa_607**	Raakow	13.631	53.348	83	3917	260.5	SO	FR	0	C	105.9	Very_low	5	1.4	28	0.8
29	**Rit_258**	Rittgarten	13.707	53.382	71	4287	281.5	SO	ER	0	R	102.1	Low	10	2.0	32	1.7
30	**Rit_259**	Rittgarten	13.707	53.384	72	1046	117.7	SO	ER	5	R	94.5	Medium	11	1.9	20	1.5
31	**Rit_265**	Rittgarten	13.704	53.378	69	3626	254.2	SO	ER	0	C	134.6	Medium	12	1.9	33	2.1
32	**Rit_259**	Rittgarten	13.701	53.377	70	6034	308.9	SO	ER	0	M	80.3	None	7	1.8	18	1.1
33	**Rit_275**	Rittgarten	13.709	53.386	72	4402	254.9	SO	ER	0	C	61.3	Low	15	1.8	42	1.5
34	**Rit_287**	Rittgarten	13.699	53.386	74	2528	210.2	S	EW	20	R	155.5	Very_low	10	2.2	33	1.7
35	**Wil_235**	Wilhelmshof	13.722	53.328	63	1447	148.8	S	FR	25	R	101.6	Medium	9	1.8	29	2.0
36	**Wil_236**	Wilhelmshof	13.721	53.328	62	441	79.1	S	FR	0	R	104.5	None	9	1.6	13	0.6

Table C4. Details for the model selection based to test the effect of number of kettle holes occurring at different radii (degree of isolation) on bee species richness and abundance. Models follow a Poisson distribution and selection based on AIC and Explained Deviance (ExplDev). Degree of isolation alone did not show any effect on species richness nor abundance, but the interaction of area of the kettle hole (patch size) had an effect on species richness and abundance at 1000m and 200m buffer, respectively. Best model was selected based on AIC (lowest value) and Explained Deviance (highest value) criterion.

Model description							**Species richness**				**Abundance**			
							glmID	**ExplDev**	**adjExplDev**	**AIC**	**glmID**	**ExplDev**	**adjExplDev**	**AIC**
0	m1p	*x*	~	1			b0p	0.00	0.00	215.70	b0p	0.00	0.00	1135.50
3	m3p	*x*	~	Buffer_100			b3p	0.00	-0.03	217.60	b3p	0.01	-0.02	1130.38
4	m4p	*x*	~	Buffer_200			b4p	0.01	-0.01	216.68	b4p	0.05	0.02	1090.25
5	m5p	*x*	~	Buffer_500			b5p	0.00	-0.02	217.40	b5p	0.01	-0.02	1126.17
6	m6p	*x*	~	Buffer_1000			b6p	0.00	-0.03	217.52	b6p	0.04	0.01	1101.04
7	m7p	*x*	~	Buffer_2000			b7p	0.00	-0.03	217.62	b7p	0.04	0.01	1098.50
8	m8p	*x*	~	Log_area	*	Buffer_2000	b8p	0.08	0.00	216.43	b8p	0.13	0.05	1020.49
9	m9p	*x*	~	Log_area	+	Buffer_2000	b9p	0.00	-0.05	219.43	b9p	0.04	-0.01	1097.69
10	**m10p**	***x***	**~**	**Log_area**	*****	**Buffer_1000**	**b10p**	**0.14**	**0.06**	**212.34**	b10p	0.17	0.10	980.53
11	m11p	*x*	~	Log_area	+	Buffer_1000	b11p	0.01	-0.05	219.35	b11p	0.04	-0.01	1101.03
12	m12p	*x*	~	Log_area	*	Buffer_500	b12p	0.07	-0.02	217.10	b12p	0.20	0.13	949.58
13	m13p	*x*	~	Log_area	+	Buffer_500	b13p	0.01	-0.05	219.23	b13p	0.01	-0.04	1125.83
14	**m14p**	***x***	**~**	**Log_area**	*****	**Buffer_200**	b14p	0.03	-0.06	219.94	**b14p**	**0.22**	**0.16**	**928.09**
15	m15p	*x*	~	Log_area	+	Buffer_200	b15p	0.02	-0.04	218.39	b15p	0.06	0.00	1085.76
16	m16p	*x*	~	Log_area	*	Buffer_100	b16p	0.08	0.00	215.97	b16p	0.13	0.05	1021.12
17	m17p	*x*	~	Log_area	+	Buffer_100	b17p	0.01	-0.05	219.29	b17p	0.01	-0.04	1125.83
18	m18p	*x*	~	Log_area	*	Buffer_50	b18p	0.09	0.01	215.19	b18p	0.06	-0.02	1085.58
19	m19p	*x*	~	Log_area	+	Buffer_50	b19p	0.09	0.04	213.21	b19p	0.06	0.00	1084.84
20	m20p	*x*	~	Log_area			b20p	0.09	0.01	218.86	b20p	0.06	-0.02	1062.62

Table C5. Definitions of bees functional traits taken from Kratschmer et al. (2019).

Trait	**Type**	**Definition**
Sociality	Parasitic	Females lay eggs in nests of specific hosts. Less efficient pollinators.
	Solitary	Females alone establish their own nest and resource collection.
	Eusocial	Living in society. Division of tasks: egg-laying and that resource collection.
Lecty	Oligolectic	Pollen specialists: pollen collected from a single or related plant taxa. Morphological adaptation to flower structure
	Polylectic	Pollen generalists: pollen collected from different plant taxa. Visits to a great variety of flowers.
Nesting type	Ground nesting	Nests are formed excavating in the ground.
	Above-ground nesting	Nests are stablished in pre-existing cavities (e.g. plant stems, dead wood, etc.).
Body size		The shortest linear distance measured between a wing tegulae across the dorsal thorax. Strongly correlated with flying distance.

Fig. C1. Color traps used to sample bees in the kettle holes. Each trap had 6 plastic containers 2 white, 2 yellow and 2 blue arranged randomly filled with water and 3–4 drops of dishwashing detergent. We positioned four sets of colour traps in each kettle hole.

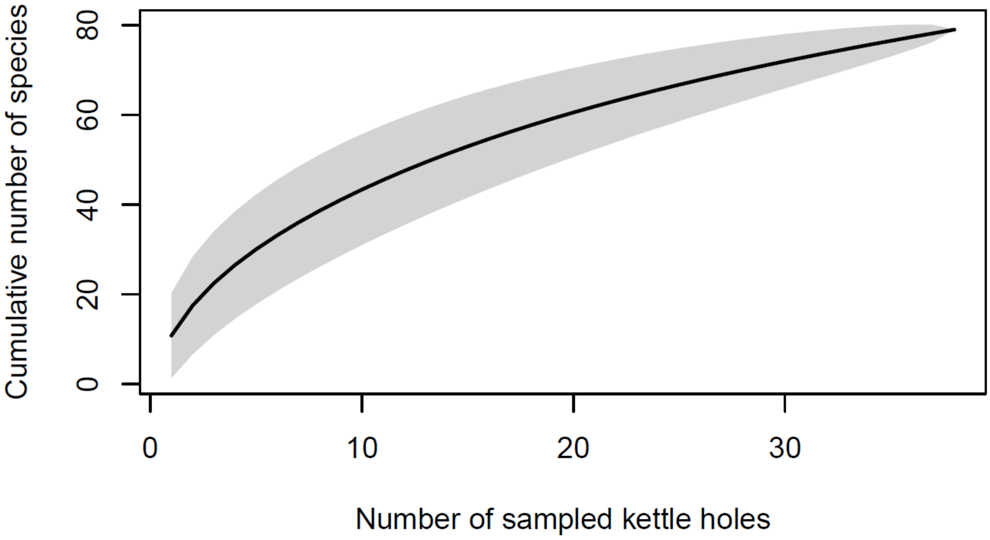


Fig. C2. Rarefaction accumulation curve of species based on 9999 permutations. Due to the curve is approaching the "plateau", we can assume that bee species collected in a total of 36 kettle holes is reliable.

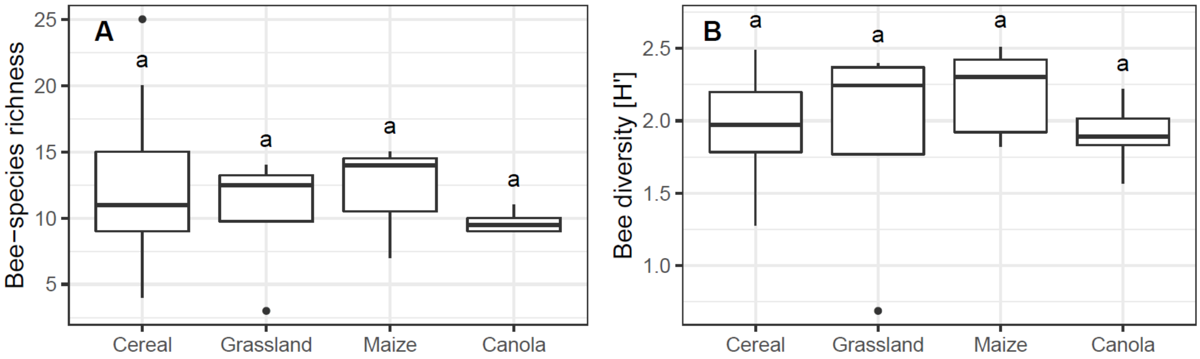


Fig. C3. Wild-bee diversity according type of cropland. No difference was found in species richness (A) or Shannon index (B) respect to type of cropland (cereal, maize, canola) surrounding the kettle hole, including grassland. Letters represent differences according post-hoc Tukey test.

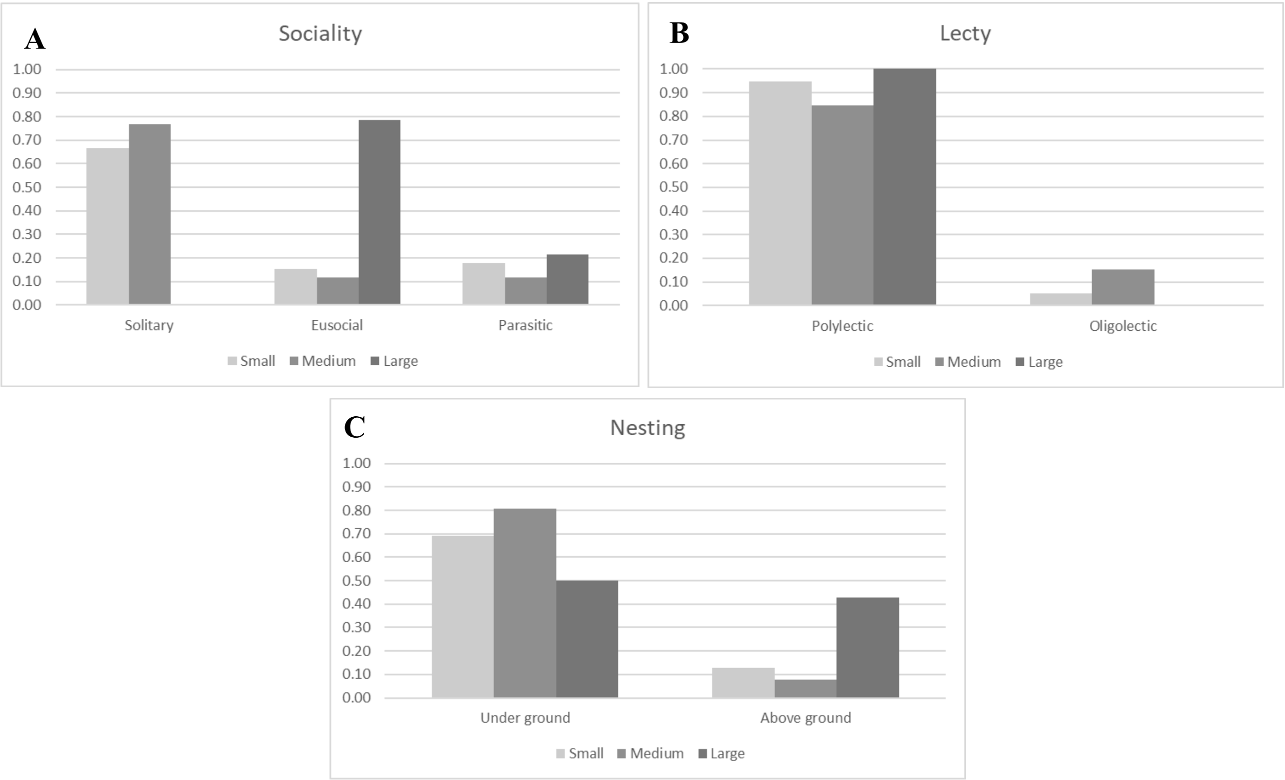


Fig. C4. Proportion of small, medium and large species according functional traits. Most small and medium sized species are solitary, while most of eusocial are large (**A**). Polylectic species are the most abundant independent of the size with complete lack of oligolectic large species (**B**). Species that have nesting underground are most abundant in small and medium sized species while in large above- and underground nesting have similar proportion (**C**).

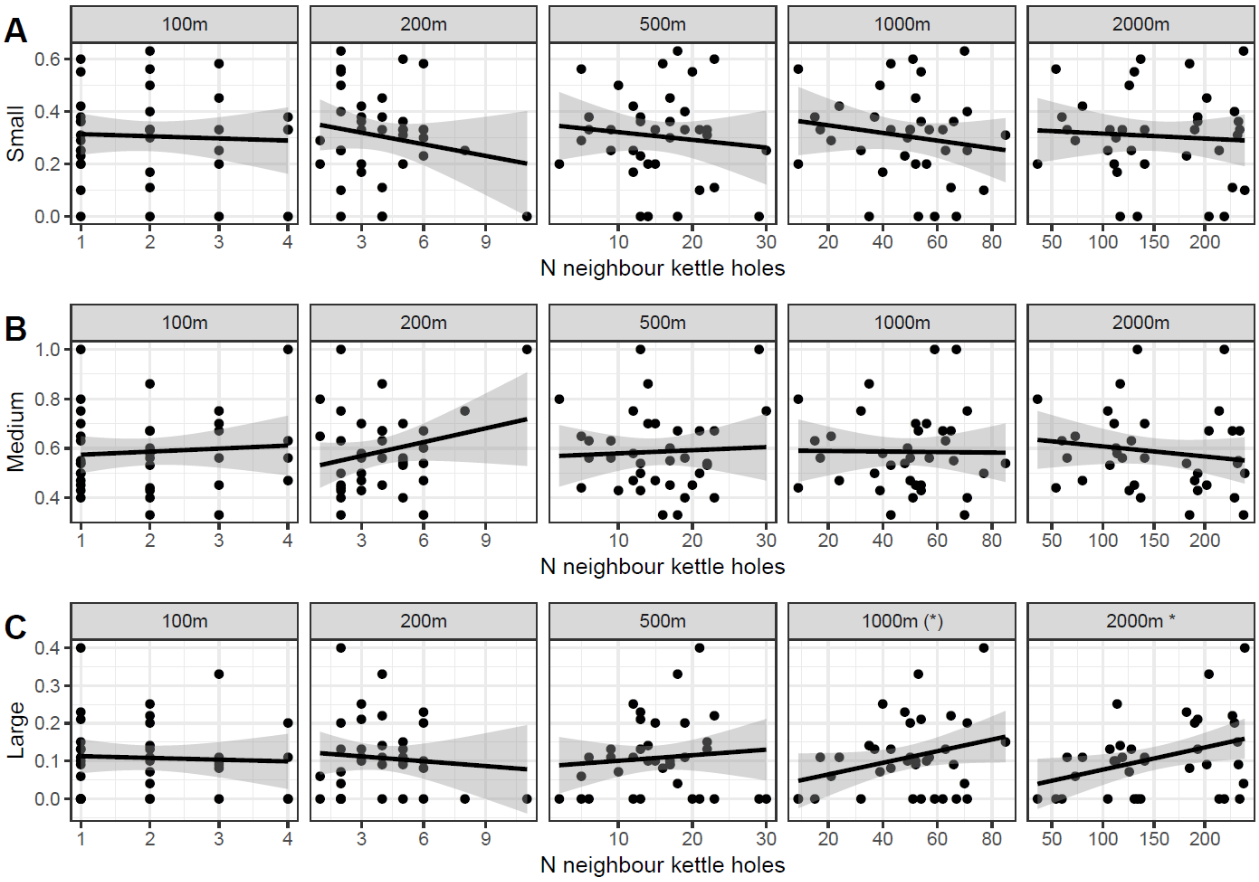


Fig. C5. Relationship between bee species richness according to body sizes with degree of isolation. Total number of species per kettle hole was calculated according to three groups: Small, medium and large. We found a positive correlation only at 1000 and 2000m for large individuals. Significance levels are indicated with asterisks: *P<0.05, (*) P=0.07.

Acknowledgements

First of all I would like to thank my supervisors Jasmin Joshi and Ralph Tiedemann for their support and motivation during the last three and a half years. I am very grateful to both of them for their suggestions, great discussions and very fast responses.

A big thanks goes to Florian Jeltsch for setting the roots of the BioMove Graduate School and all the professors and PIs involved. I thank all the BioMovers for all the experiences that we shared in the last years. I benefited a lot from our BioMove Fridays, other meetings, retreats, seminars and workshops. Thanks to all the people involved in the project for all the enthusiasm and commitment. And to DFG for funding that allowed me to fulfill this research and gave me the opportunity to participate in several conferences and summer schools.

I am very grateful with to the people in Dedelow, who helped a lot with the logistics in the field. Special thanks goes to Frenze and Jette Geiger for making the hard-working days in the field so enjoyable. To Carlos Acame who helped me and thought me how to use a drone for making pictures, and also helped me sampling in the kettle holes. I want to extend a big thanks to Gabriela Onandia, Camille Musseau, Trupti Bhamre, Benito Schöpke, Sebastian Hausmann, and all people that helped me at some point in the field.

I am also very grateful with Sandra Jönsson for her guidance and support in the laboratory. Thanks to Katja for help with microsatellites. To Marco Donat for his work in the laboratory that ended in a very successful master project. Special thanks to Christian Schwarzer for teaching me how to analyze population genetics data and for sharing his experience in the laboratory. And to Dorit Siebert and Florian Fahr for help preparing the bees.

Thanks to all Phd students, postdocs and technicians from AG Joshi and AG Tiedemann. Thank you to Sofia and Katrin for being great office roommates, for all the discussions, help in writing and coffee breaks. Thanks to Julia and Katrin for helping me with the German abstract. Thanks to Susanne Stang, Larissa and Wiebke for helping me with statistics and GIS. I am very grateful to all my coauthors that contributed and improved the quality of the manuscripts. And special thanks to Federico Luebert and Gianluigi Ottaviani for voluntary revising and correcting the introduction and discussion of this thesis.

Special thanks to Pierluigi Colangeli for being a great friend and for helping me with statistics and R. A big thank to Magdalena Litwin for her lovely friendship and always ready kind words to overcome any problem. And a big thank you to Carlos Landivar, for his motivation and positive energy.

Finally I huge thank you to my dad Blithz, my stepmother Erika and my brother Lucas for all their positive energy from across the globe. For all the sweet talks and funny messages. Thank you for all the encouragement, endless love and support. ¡Muchas gracias!

And finally I am immensely grateful with my sweet love Rok Cestnik, for all his help with ideas, creative ways to analyze data and very helpful discussions. Thank you so much for your patience, dedication and love. Hvala!

Declaration of Authorship

I hereby declare to have prepared this dissertation independently under the commonly accepted help of my supervisors. All direct or indirect sources used are given as references. All contributions of co-authors are acknowledged. This thesis has not been submitted to any other university or institution before.

Potsdam, 26th June 2019

Sissi Donna Lozada Gobilard