Flooding in a grassland diversity experiment:

response of microorganisms, microarthropods

and predator – prey interactions

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Odette González Macé

aus

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Betreuungsausschuss

Stefan Scheu and Mark Maraun,

J.F. Blumenbach Institute of Zoology and Anthropology; Göttingen University

Mitglieder der Prüfungskommission

Referent: Stefan Scheu

Korreferent: Mark Maraun

Weitere Mitglieder der Prüfungskommission:

Marko Rohlfs, Population and Evolutionary Ecology, Bremen University

Christoph Bleidorn, Animal Evolution and Biodiversity, Göttingen University

Klaus Hövemeyer, Zoology and Anthropology; Göttingen University

Nico Eisenhauer, Experimental Interaction Ecology, Leipzig University

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To forget how to dig the earth and to tend the soil is to forget ourselves

Mahatma Gandhi

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Summary

Flooding frequency is predicted to increase during the next decades. This will need a better understanding of flood impacts on terrestrial ecosystems and the development of strategies to mitigate potential damage. Plant diversity is expected to buffer flooding effects by providing a broad range of species responses. However, little effort has been made to synthesize soil responses to disturbances by flooding and the majority are simulated floods under greenhouse or field conditions. In contrast to these limited investigations I collected data information from a major natural flood occurred in 2013 to investigate the soil food web responses to flooding. My study took place in the context of one of the longest-running biodiversity experiments worldwide. I investigated three compartments of the aboveground-belowground food web: microorganisms (Chapter 2), microarthropods (Chapter 3) and predator-prey interactions (Chapter 4).

In Chapter 2 I analized the flood-induced changes in soil microbial functions as modified by plant diversity. In the short-term, microbial biomass was reduced in the severely flooded plots at high plant functional group richness. Flooding alleviated microbial nitrogen limitation, presumably due to the input of nutrient-rich sediments. However, carbon and phosphorus limitation was greater in higher diversity plots and at higher flooding index. Furthermore, the activity of soil enzymes (including 1,4-β-N-acetylglucosaminidase, phenol oxidase and peroxidase) increased with flooding index. These results suggest a greater chitin and lignin degradation as a consequence of the input of detritus in sediments. Carbon and phosphorous were less limited in the medium-term than in the short-term but all the functions of the soil microbes rapidly recovered.

In Chapter 3 I explored the response of Collembola and Acari communities to the flooding. Collembola and Acari densities and Collembola and Oribatida species richness were greatly affected by the flood but recovered within three months. The effects of plant community composition on soil microarthropods disappeared after the flood, presumably due to homogenization of the field by the flood. However, the effects of plant community were being reasserted three months after the flood. Mobile, surface-living soil animals such as Entomobryidae (Collembola) and Prostigmata (Acari) recovered most rapidly and some species took advantage of resources (i.e., dead plant material) brought by the flood. Widespread microarthropod species with wide habitat niches recovered more rapidly than those with more
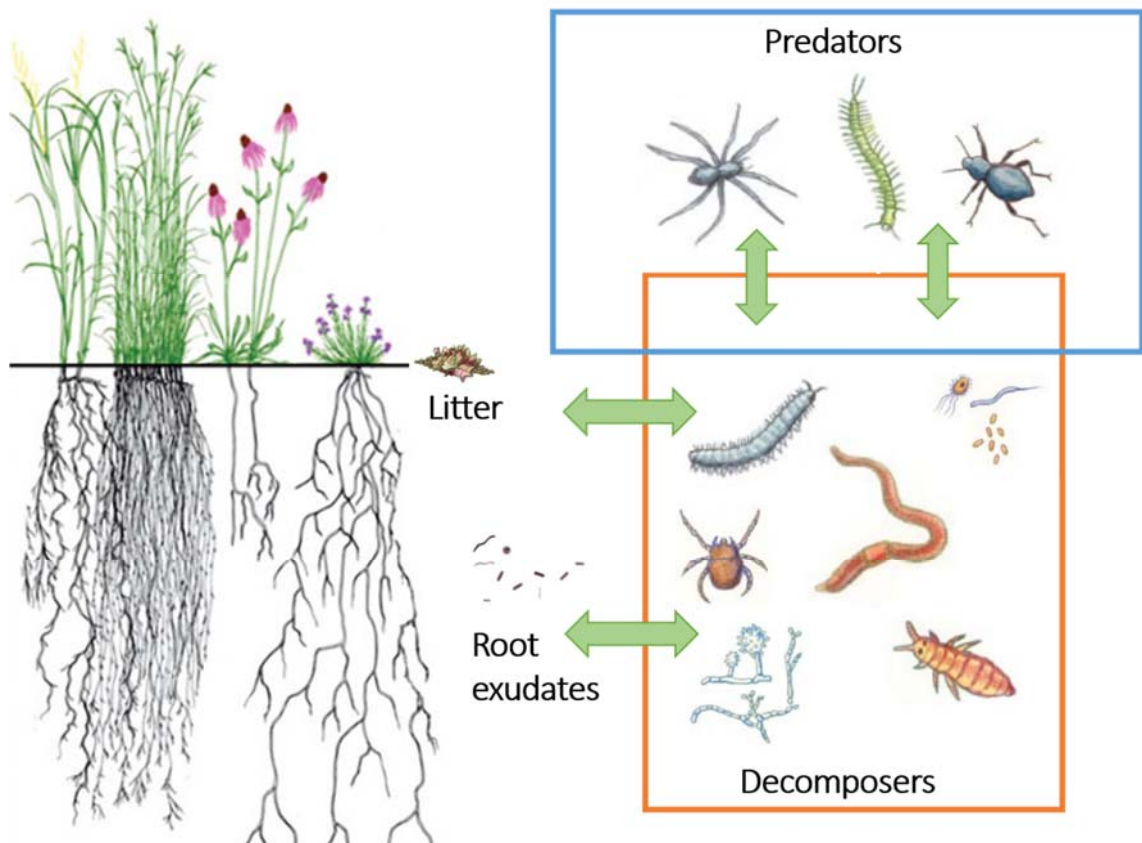
limited distributions and narrower niches, resulting in moderate to small changes in microarthropod community composition.

In Chapter 4 I used fatty acid and stable isotope techniques to examine variations in the trophic niches of two generalist predator species: the ground beetle *Harpalus rufipes* and the wolf spider *Trochosa ruricola*. The diet of *H. rufipes* differed between plant species diversity and plant functional group. In the presence of grasses and small herbs *H. rufipes* ate more seeds. In the presence of legumes they ate a greater proportion of animal prey, presumably aphids or collembolans. In contrast, the diet of *T. ruricola* was limited to animal prey and varied mainly with the spider body size. Larger individuals occupied a higher trophic position in the food web. Moreover, the diet of *T. ruricola* changed due to flooding, presumably because more secondary decomposers and intraguild prey were available in severely flooded plots. My results suggest that, consumers at higher trophic positions in the soil food web responded partially to the flood. Probably, generalist predators are well adapted to disturbances due to their wide food spectrum.

Overall, my thesis highlights the rapid recovery of the soil food web after a severe flooding. Although the effects of the 2013 flood were lower than expected, the communities were significantly affected in the short-term. However, the rapid recovery of plants and inputs from nutrient-rich sediments generated a quick response in the soil organisms. These findings support that grasslands are disturbed habitats and their food webs seem to be well adapted to disturbances. Moreover, since pulse disturbances are part of all ecosystems, they help to maintain diversity and mosaic landscapes and contribute to large scale ecological resilience.

Chapter 1

General Introduccion

General Introduction

Recent global change scenarios predict that the incidence of disturbance events will increase in the future (Knapp et al., 2008). However, large and infrequent natural disturbances are little studied due to the lack of experimental control over them (Michener & Haeuber, 1998). I investigated a unique 200-year flood event in central Europe. This event provided an opportunity for exploring the response of the soil food web to flooding. The study formed part of the Jena Experiment, a large biodiversity experiment established on the floodplain of the Saale river close to the town of Jena (Thuringia, Germany). I looked into the response of a grassland **aboveground-belowground food web** with different plant diversity to a severe **disturbance** such as flooding. I investigated three compartments of the food web: microorganisms, microarthropods and predator-prey interactions.

Aboveground-belowground food web

Terrestrial ecosystems can be divided into an aboveground and belowground subsystem. These subsystems are tightly linked and affect each other simultaneously because they are connected by plants and organisms living in both compartments (Bardgett et al., 2005a; Schröter et al., 2004; Wardle et al., 2004). The subsystems affect each other in both directions, top-down from aboveground to belowground and bottom-up from belowground to aboveground.

Top-down forces arise when aboveground organisms such as generalist predators or plants control the belowground subsystem (Haddad et al., 2009; Scheu, 2001). Generalist invertebrate predators such as spiders, staphylinids and carabids link these subsystems by inhabiting both in different life-stages and feeding in both as adults (Berg et al., 2008; Scheu, 2001). In addition to predators, plants also affect the belowground community (Fig. 1). Plants interact with soil organisms in many ways because a large part of the plant biomass is located belowground (Jackson et al., 1996; Mokany et al., 2006). Plants deliver organic materials including labile compounds secreted by roots and litter residues to the belowground subsystem (Albers et al., 2006; Ostle et al., 2007). The quality and quantity of organic matter inputs, as well as the changes in microenvironmental conditions, stabilize the populations of soil organisms (Milcu et al., 2010) and control the composition and functioning of soil communities (Eisenhauer et al., 2010; Lange et al., 2015; Nilsson et al., 2008). Moreover, the greater the diversity of plant derived resources the greater the diversity of decomposer microorganisms, detritivores and herbivores in soil. The high decomposer diversity, in turn, promotes the diversity of other components of the soil food web (Hooper et al., 2000; Scherber et al., 2010). Plant functional group also affects the

belowground community. For example, decomposer organisms benefit from the high quality litter provided by legumes (Milcu et al., 2008; Spehn et al., 2000). However, the relationship between plant diversity and the diversity of soil organisms remains controversial. Long-term studies found them to be closely linked (Eisenhauer et al., 2011; Sabais et al., 2011; Viketoft et al., 2009) whereas some studies found the relationships to be weak or non-existent (Bardgett & Wardle, 2010; Vogelsang et al., 2006). Consequently, more research on this topic is needed.
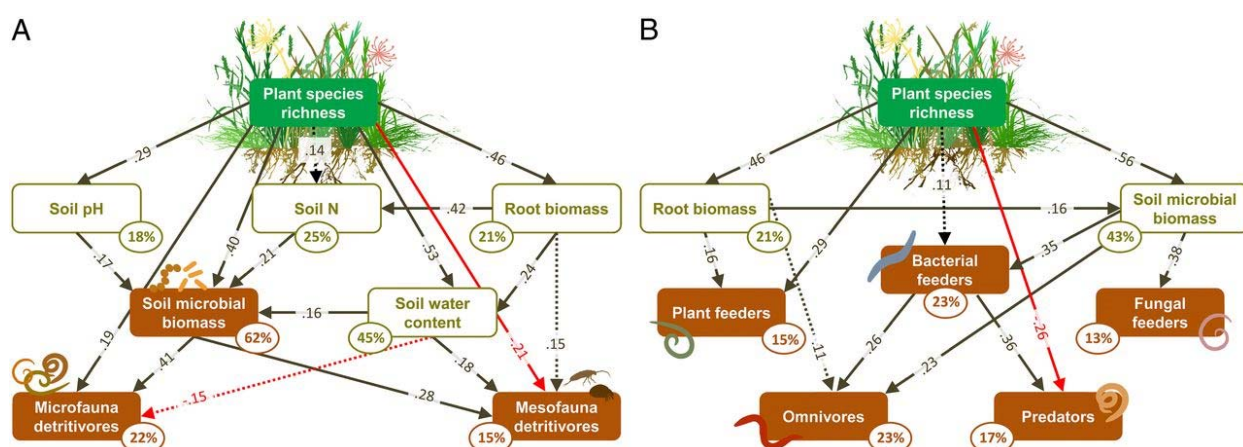

Fig. 1. Path analysis models of plant diversity effects on soil biota. (A) Causal influences of plant diversity (exogenous variable; green rectangle) on soil pH, soil N concentration, soil water content, root biomass productivity (endogenous explanatory variables; white rectangles), microbial biomass, and abundance of soil animals (endogenous variables; brown rectangles; P = 0.93). Numbers on arrows are standardized path coefficients. Solid line arrows indicate significant paths (P < 0.05); dotted lines indicate non-significant arrows (black = positive, red = negative). Percentages indicate the variance explained by the model. (B) Causal influences of plant diversity on the soil nematode community (P = 0.24). Figures are from Eisenhauer et al. (2013).

Bottom-up forces arise when belowground animals affect plant performance and aboveground communities (Scherber et al., 2010). Soil organisms influence decomposition processes and nutrient cycling, thereby affecting nutrient acquisition and growth of plant (Bardgett & Chan, 1999; Wardle et al., 2004). In consequence, a cascade effect may trace from belowground organisms to herbivores and higher aboveground trophic levels (Eisenhauer & Scheu, 2008; Wurst, 2010; Wurst & Jones, 2003). Moreover, the soil biota forms antagonistic (herbivores, pathogens) or mutualistic (mycorrhizal fungi, plant growth promoting rhizobacteria, rhizobia) associations with plants (Wardle et al., 2004). Changes in these relationships can modify competition (Wardle et al., 2004), community structures (Bonkowski & Roy, 2012) and succession dynamics of plant (de Deyn et al., 2003). In consequence, these interactions regulate ecosystem

processes such as soil respiration (Coleman & Whitman, 2005; Heemsbergen et al., 2004), litter decomposition (Cornwell et al., 2008; Heemsbergen et al., 2004) and also global processes such as carbon cycling (Schlesinger & Andrews, 2000). In addition, the soil biota drives both positive and negative complementarity between different plant species via multiple mechanisms (Eisenhauer, 2012). On the positive side, soil animals can enlarge biotope space, mediate legume effects, increase plant community resistance and maintain plant diversity. But, on the negative side, the soil biota can reinforce negative complementarity effects by competing with plants for nutrients or by exerting herbivore or pathogen pressure, thereby reducing plant productivity. As a whole, the organisms of both subsystems coexist and interact with each other. They form a complex food web with plants as intermediaries (Bardgett, 2005a; Smith et al., 2015; Wall et al., 2010).

Plants supply the energy of the soil food web system through two channels: litter materials and root-based resources. In grasslands, the aboveground biomass is removed as plant yield, therefore root exudates and other root-derived resources are important. Soil microorganisms are the base of the soil food web. They are responsible for many ecologically and economically key ecosystem functions (Bardgett & van der Putten, 2014; Bodelier, 2011; Philippot et al., 2013) such as decomposition, nutrient cycling, primary productivity and climate regulation (Bardgett & van der Putten, 2014; Nemergut et al., 2014; Schimel & Schaeffer, 2012). They mediate in 80–90% of the processes in soil (Coleman et al., 2017; Nannipieri & Badalucco, 2003). Soil microorganisms are assumed to be limited predominantly by carbon but other elements, in particular nitrogen and phosphorus, are also important regulatory forces (Demoling et al., 2007). Carbon in belowground systems is embedded in complex and recalcitrant compounds, such as lignin and humic acids that are not easily available for decomposers. Soil microorganisms use an array of extracellular enzymes to degrade these compounds (Burns et al., 2013). Furthermore, a portion of plant-derived carbon enters the belowground system via living roots (Albers et al., 2006; Paterson et al., 2011; Pollierer et al., 2007). This route provides low molecular weight compounds, such as sugars, organic acids and amino acids that are easily available to soil organisms (Bertin et al., 2003). This labile fraction of litter and roots is predominantly used by bacteria rather than fungi. The fungi tend to use the more recalcitrant compounds of organic matter (Lundquist et al., 1999; Paterson et al., 2008).

After being incorporated into soil microorganisms, carbon and other nutrients propagate to higher trophic levels. Soil food webs typically contain several trophic levels forming a gradient from organisms predominantly feeding on detritus (primary decomposers) to those

predominantly feeding on microorganisms (secondary decomposers) and those on the other animals (predators) (Eissfeller et al., 2013; Scheu & Falca, 2000). Primary decomposers, such as Diplopoda, Annelida and some groups of Oribatida (Acari), predominantly rely on plant litter and plant derived dead organic matter as food resources (Eissfeller et al., 2013; Pollierer et al., 2009; Scheu & Falca, 2000). Secondary decomposers, such as most Oribatida and Collembola species, predominantly feed on microorganisms and microbial residues. They are the most widespread and abundant soil arthropod groups (Bardgett, 2005b; Petersen & Luxton, 1982) and live in almost all terrestrial habitats. Oribatida and Collembola contribute to decomposition processes and nutrient cycling (Chamberlain et al., 2006; Krantz & Walter, 2009). Although Collembola and Oribatida are often grouped into the same trophic level and are considered to occupy similar niches in decomposition processes (Kaneko et al., 1995; Wallwork, 1970), the two groups differ in their ecological traits including mobility, reproduction, level of predation pressure and tolerance to abiotic conditions (Lindberg & Bengtsson, 2005; Maraun et al., 2003a; Siepel, 1994).

The trophic level after secondary decomposers is formed by predators. Predatory soil arthropods, such as Mesostigmata (Acari), Chilopoda, Araneae and Carabidae (Coleoptera) are assumed to be food generalists (Scheu & Falca, 2000; Scheu & Setälä, 2002). From a community perspective generalist predators living belowground are important control agents of insect herbivores aboveground (Romero & Harwood, 2010; Wise, 1993). Araneae and Carabidae occupy different niches and complement each other in prey population control. Carabidae are opportunistic generalist predators (Gallandt et al., 2005; Harrison et al., 2003; Lee et al., 2001), with some of them consuming substantial amounts of plant seeds (Harrison et al., 2003; Lund & Turpin, 1977). Thereby, they not only act as antagonists of insect pest species but may also contribute to weed suppression (Gallandt et al., 2005; Kulkarni et al., 2015; Menalled et al., 2006). Like Carabidae, Araneae can also reach high levels of local species richness and abundance (Diehl et al., 2012; Malumbres-Olarte et al., 2013) and can thereby effectively control insect herbivore pest species (Sunderland, 1999). Their wide prey spectrum allows them to occupy a variety of niches (Wise, 1993). In addition to prey availability, physical habitat characteristics, such as plant architecture, may also determine species diversity and composition of predatory communities in the soil (Langellotto & Denno, 2004; Uetz, 1991).

<u>Disturbance</u>

Natural disturbances such as wind, flood, drought and fire have shaped ecosystems and organisms within the biosphere for millennia, influencing the structure and functioning of ecosystems and the distribution patterns of organisms (Grime, 2006; Lytle, 2001; Southwood, 1988). Disturbance is a crucial driver of ecological processes. It represents any discrete event in time that disrupts ecosystem, community or population structure and changes resource availability or the physical environment (Pickett & White, 1985). In habitats subjected to disturbance, the distribution of species is determined both by the degree to which species survive disturbance events and the extent of their recovery in the periods between such events, as influenced by dispersal, recruitment and competition between and within species. Direct responses of species to disturbance are crucial because surviving organisms not only determine the short-term post-disturbance patterns but also dictate much of the successional trajectory between disturbances (Turner et al., 1998). Understanding the effects of disturbance on population dynamics is critical for predicting species abundance and persistence over time. Disturbances such as floods and fires impose high mortality via movement of substrates and habitat destruction. Nevertheless, they can also positively affect population growth by regenerating physical habitats, enhancing the availability of food resources and maintaining physical linkages between adjacent habitats such as rivers and their floodplains (Effenberger et al., 2006; Junk et al., 1989; Power et al., 1996).

For many years, ecologists have attempted to predict how ecosystems will change after disturbances. Many hypotheses have been proposed to predict or explain successional pathways and patterns of biological diversity. However, no theory has been universally agreed upon. This is, in part, due to the complexity and variability of the ecosystems and disturbance events involved (Kayes et al., 2010). Succession was among the first theories developed in ecology. Ecological succession is the process of change over time in species composition of an ecological community. Succession splits into primary succession and secondary succession. Primary succession occurs in areas where there is not existing life soil structure such as of new emerged land, e.g. dunes or lava flows (Campbell et al., 2006; Clements, 1916; Cutler, 2011; Fig. 2A). Otherwise the process is secondary succession (Fig. 2B). Because very few disturbances result in the removal of all life (Connell & Slatyer, 1977) most disturbances initiate secondary succession. In this case the soil is intact and frequently contains large number of germules from before the disturbance (Campbell et al., 2006; Clements, 1916). Nevertheless, the traditional distinction between primary and secondary succession is insufficient to capture the tremendous variability

in succession following large and infrequent disturbances (LIDs; Turner et al., 1998). The spatial variability within LIDs often leads to a heterogeneous pattern of surviving organisms. This variety of conditions results from preexisting site factors and spatial variation in intensity of the disturbance itself. The abundance and spatial arrangement of such *biological legacies* or *residuals* can have substantial influences on successional dynamics after disturbances (Franklin et al., 1985; Michener et al., 1998; Del Moral, 1998;). So called "lifeboat" organisms can influence subsequent recruitment of colonists through ecosystem engineering or interspecific interactions, maintain species diversity and structural complexity and help to re-establish ecosystem functions (Franklin et al., 2000; Ledger et al., 2006; Swanson et al., 2010). When the density of survivors is high, residuals can negatively affect colonists due to competition whereas, at low survivor densities residuals can enhance colonization by ameliorating harsh abiotic conditions (Bertness & Callaway, 1994). LIDs create more variability in successional pathways than smaller disturbances do and can thus provide unusual opportunities for the initiation of multiple stable states thereby enhancing the diversity of communities across the landscape.

One of the first theories about ecological succession was developed by Clements (1916). He conceptualized succession as occurring in a directional and predictable manner, commencing from a "bare state" and progressing from pioneer species to a "climax" or final stage. Succession was perceived as a series of invasions, starting with pioneer species, with each stage in turn being invaded by a "higher" form (Clements, 1916). Established species alter environmental conditions so that they are less favorable for themselves and potentially more favorable to species from the next stage. This occurs until conditions are most favorable to the current set of species and the climax stage is achieved (Clements, 1916). The idea of a climax or final equilibrium state was central to Clements' ideas but today ecologists generally disagree with this idea and view systems as dynamic, complex and nonequilibrial in nature (Connell & Slatyer, 1977; McIntosh, 1999; Moore et al., 2009).
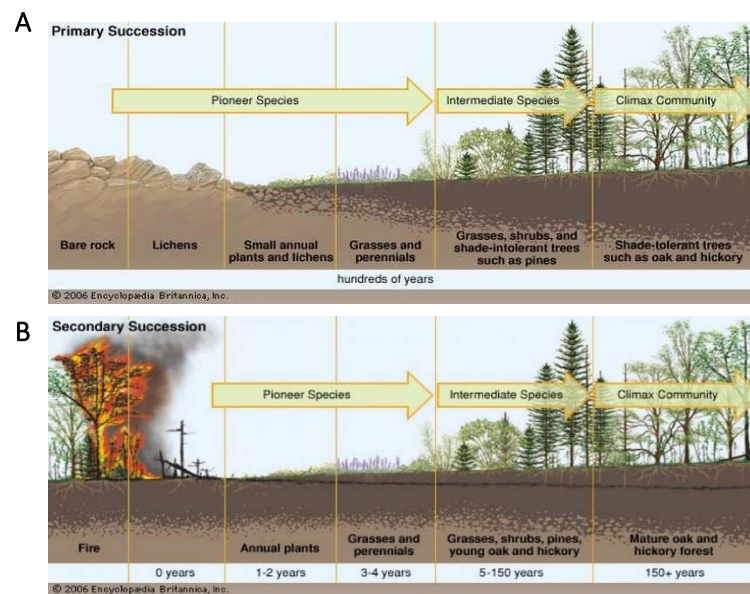
Fig. 2. (A) Primary succession. It begins in barren areas. Afterwards lichens or plants can survive in this environment. Over hundreds of years these "pioneer species" convert the rock into soil. Each successive stage modifies the habitat. The final stage of succession is a climax community. (B) Secondary succession. It follows disturbance and begins in environments that already possess soil. The stages of secondary succession are similar to those of primary succession. Figures are from Encyclopædia Britannica, Inc.

Another well-established theory is the Intermediate Disturbance Hypothesis (IDH) (Connell, 1978; Pickett & White, 1985; Wootton, 1998). IDH predicts change in species richness in relation to disturbances (Connell, 1978; Grime, 1973; Fig. 3). Disturbances vary in frequency, extent, intensity, duration or time since a previous disturbance (Shea et al., 2004). At low levels of disturbance, the most competitive species come to dominate. At high levels of disturbance, only extremely resistant species or rapid colonisers will manage to reach maturity between disturbances (Connell, 1978; Wilson et al., 2012b). At intermediate levels of disturbance, more species may coexist due to low competition, varying rates of response to resource availability or a competition–colonisation trade-off (Shea et al., 2004). IDH has been criticized for this assumptions and limitations (Fox, 2013). It assumes a strong degree of biotic interaction while ignoring abiotic factors (Reice, 1984). For example, it does not consider how fire increases nutrient cycling and so favours specialists such as legumes (Abrams & Dickmann, 1983; Masters, 1993). It assumes interactions on a single trophic level and views organisms on other trophic levels as being non-influential (Wootton, 1998). However, disturbances may temporarily relieve the affected trophic level from competition and cause intensified competition elsewhere in the food web (Barnes & Minshall, 1983). For example, a disturbance at a low trophic level results in less competition at that level and subsequent prey shortage at high trophic levels (Barnes & Minshall, 1983; Reice, 1984). Finally, mobile organisms are more likely to flee or seek refuge

during a disturbance and later return to the disturbed habitat. These populations generally do not suffer substantial losses and therefore do not respond as IDH suggests.
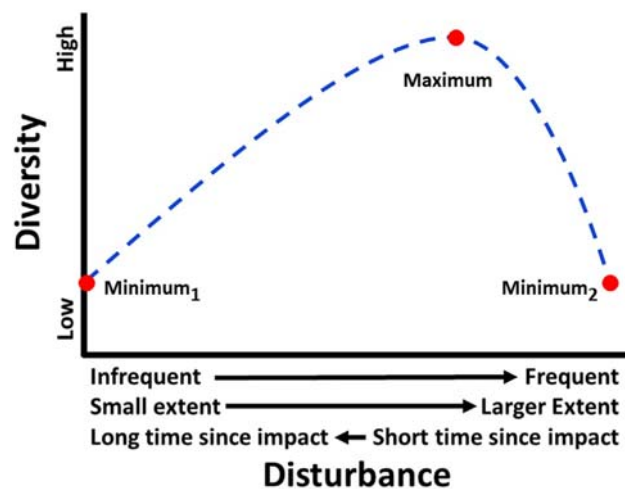

Fig. 3. Simplified graphical representation of the Intermediate Disturbance Hypothesis, which posits that diversity should be highest at intermediate levels of disturbance, and lowest at either the highest (minimum2) or lowest (minimum1) end of the disturbance gradient, depending on the characteristics of habitat represented by the extremes. In its incarnation by Connell (1978), the disturbance gradient (x-axis) could represent either the frequency or extent of a disturbance type, or the time since impact of a particular disturbance event. Figure is from Willig and Presley (2018).

Flooding is one type of frequent disturbance that affects streams, rivers and their terrestrial surroundings. Intense precipitation events are expected to increase in many parts of the world in the near future (Hirabayashi et al., 2013; IPCC, 2013; Jongman et al., 2014) and therefore the risk of stream and river flooding is projected to rise (Dankers & Feyen, 2009; Hirabayashi et al., 2013). Floods will affect nutrient and sediment dynamics with potentially drastic consequences for vegetation and fauna (García et al., 2014; Merritt et al., 2010; Poff, 1997). Soil structure and fertility also are likely to be affected and nutrient availability is reduced with an initiation of primary succession processes in the case of strong disturbances (Isbell et al., 2015). However, moderate floods also may beneficially affect nutrient cycling and increase habitat heterogeneity, thereby fostering biodiversity (Isbell et al., 2015).

Despite the importance of soil organisms to ecosystem functioning (Coleman et al., 2004; Fierer et al., 2007; Wall & Bardgett, 2012) little effort has been made to gain insight into soil food web responses to flood disturbances. Floods limit soil gas diffusion and oxygen availability. They also promote the mineralization and decomposition of dead organic material (Schuur & Matson, 2001) fostering anaerobic conditions in soil (Unger et al., 2009). Such changes can alter soil community composition and soil food webs (Wagner et al., 2015; Wu et al., 2015). Changes in the structure and functioning of soil communities after flooding may be due to greater resource

availability or environmental selection for certain functional types. Zhou et al. (2002) reported that soils saturated in water have reduced bacterial diversities. The traits of organisms can affect how they are affected by flood. Large body size or dormancy in the egg stage can promote survival and highly mobile species can rapidly immigrate into sites and so recolonize them after flooding (Lambeets et al., 2009; Rothenbücher & Schaefer, 2006). For example, Oribatida species are not highly mobile, have low reproductive rates and only slowly recolonize disturbed habitats (Maraun & Scheu, 2000). Collembola, in contrast, are more sensitive than Oribatida to abiotic microhabitat conditions but recolonize disturbed habitats more quickly (Lindberg & Bengtsson, 2005; Maraun et al., 2003b). Moreover, the impact of flooding also depends on where the organisms live in the soil. Epigeic organisms, living on the surface and upper layers of soil, are immediately and severely affected by flooding, whereas endogeic species, dwelling deeper in mineral soil, are protected and so less severely affected but they also recovered more slowly. However, there is limited understanding of how short-term disturbance events affect soil biodiversity (Blankinship et al. 2011; Kardol et al. 2011). Greater understanding and appreciation of the relationships between disturbance processes, soil fauna and ecological functioning are of critical importance for maintaining ecosystems.

Plant diversity also plays an important role in how ecosystems respond to flooding, depending on the specific flooding tolerance of species (van Eck et al., 2004; Mommer et al., 2006) and the changes in resource availability induced by biodiversity (Odum et al., 1979; Wright et al., 2015). Communities with high plant diversity are often more resistant to environmental disturbances and plants in such communities can respond more positively to mild flooding events than those growing in monoculture (Isbell et al., 2015; Reich et al., 2001; Wright et al., 2015). Diverse communities have higher root biomass than monocultures (Mueller et al., 2013; Ravenek et al., 2014) and this increases belowground porosity (Fischer et al., 2015), which buffers the negative effects of flooding (Silvertown et al., 1999). However, communities with high plant diversity may be less stable than those at low diversity due to their greater biomass production at low flooding severity (Wright et al., 2015). The response can change depending on the duration of the flood. Results from a large scale field experiment demonstrated that in the short-term (three weeks after the flood), plant species in monocultures were more severely damaged by flooding than those in species-rich plant communities (Wright et al., 2017). However, in the long-term (three months after the flood), the effect on biodiversity largely depended on the plant functional groups involved. Small and tall herbs recovered to pre-flood levels but only when growing in high diversity mixtures. Grasses recovered well in both monocultures and mixtures and legumes were weakly affected in mixtures and monocultures (Wright et al., 2017).

Grassland as model system: The Jena Experiment

Grasslands in Europe have been managed increasingly intensively since the 1960s. Mown grasslands and pastures currently make up a large proportion (almost 40%) of the agricultural land in Europe (OCDE, 2011). Grassland systems are one of the most species-rich habitats (Wilson et al., 2012a). They also provide refuges for many species (Poschlod & WallisDeVries, 2002) and have essential functions for ecosystem services (Gilmanov et al., 2007; Smit et al., 2008; Soussana & Lüscher, 2007).

The Jena Experiment is a semi-natural temperate grassland experiment close to the town of Jena (50°55´ N, 11°35´ E; Thuringia, Germany; Fig. 4). It was set up in 2002 on eutric fluvisol in the floodplain of the Saale river. For at least 40 years before 2002 the site had been used as an arable field. Mean annual air temperature is 9.9°C and mean annual precipitation is 610 mm (1980-2010). The experiment comprises 80 plots, each 5 x 6 m, arranged in 4 blocks to control for changes in soil texture with distance from the river. The plots contain different plant species richness (1, 2, 4, 8, 16 and 60) and plant functional group richness (1, 2, 3 and 4) (Table 1). The plant species used were typical of Central European hayfields. To construct functional groups the species were grouped according to their morphological, phenological and physiological traits. They are grouped into grasses (16 species), small herbs (12 species), tall herbs (20 species) and legumes (12 species). The established grassland is mown twice a year and weeded three times a year. More details on the Jena Experiment are presented in Roscher et al. (2004).

Fig. 4. Photograph of the field site of the Jena Experiment taken in 2007 showing the main experimental plots varying in plant species and plant functional group richness. The field site is located on the floodplain of the Saale river at the northern edge of Jena (Thuringia, Germany; background). Photo by A. Weigelt.

Table 1. Design of the Jena Experiment. Combinations of plant species richness and plant functional group richness and number of replicates per diversity level.

		Plant species diversity						
		1	2	4	8	16	60	Total
Plant	1	14	8	4	4	2	-	32
Functional	2	-	8	4	4	4	-	20
group	3	-	-	4	4	4	-	12
richness	4	-	-	4	4	4	4	16
	Total	14	16	16	16	14	4	80

The Jena Experiment was flooded for 24 days from 30 May to 24 June 2013 (Fig. 5) due to one of the largest floods in central Europe in the past two centuries (Blöschl et al., 2013). Rainfall in May 2013 was exceptionally high, approximatively 150 mm. The flood affected much of the Jena Experiment field site and caused anaerobic soil conditions with redox potentials ranging from -121 to 193 mV in some plots (Wright et al., 2015). Water coverage was measured daily for each plot from 31 May to 24 June and assigned to 5 levels: 0, 25, 50, 75 and 100% (percentage of the plot covered by water). Flooding severity was measured using a flooding index calculated as the sum of flooded days during the whole flooding period (24 days), for details see Wagner et al. (2015).

Fig. 5. Photographs taken in 2013 in the field site when the flood invaded the Jena Experiment. Photos by Victor Malakhov.

<u>Study objectives and hypotheses</u>

In this PhD thesis I explored the effects of the summer flooding in 2013 focusing on the aboveground-belowground food web of grasslands in the framework of the Jena Experiment, including the effects of plant and functional group richness. I hypothesized that:

1. The flood which hit the Jena Experiment functioned as a large and infrequent disturbance (LID). In consequence, at least in short-term, it decreased the abundance and diversity of communities across the field and created spatial variability and heterogeneous pattern of surviving organisms.
2. Plant diversity will foster the recovery of aboveground-belowground food web.
3. Aboveground-belowground food web will be influenced drastically by the flood. Basal soil food web components will be more affected by flooding than higher trophic levels. High mobility of high trophic level species allow to find refuge more easily and to recolonize the disturbed area more quickly after the flood than basal trophic levels. Furthermore, the pattern of Collembola and Oribatida species richness will follow the Intermediate Disturbance Hypothesis. The highest species diversity occurs at intermediate levels of flooding.

In Chapter 2 I investigated the response of soil microbial functions to the flood and whether their responses were related to plant species diversity. I measured changes in microbial respiration, biomass, nutrient status as well as enzyme activity. I expected an immediate reduction in basal respiration, microbial biomass and enzyme activities due to anoxic conditions, particularly at high flooding severity. Furthermore, I expected these effects to be more pronounced in higher plant diversity plots due to high oxygen consumption by roots in more diverse plant communities with higher root biomass. Also, the flood will presumably reduce microbial nutrient limitation due to the input of nutrient-rich sediments and the enhanced availability of dead organic material. The reduction will be most pronounced at high plant diversity due to the more severe nutrient limitation via more efficient plant N capture at high plant diversity. I hypothesized that the higher the plant diversity the more rapid the recovery of microbial biomass and enzyme activities after the flood. High diversity will enhance internal nutrient cycling and higher input of root-derived residues.

In Chapter 3 I studied the effects of flood on the microarthropod community. Specifically, I explored changes in the communities of Collembola and Acari after the flood. I expected the density and richness of both groups to be greatly reduced by flooding with Collembola recovering

more rapidly than Acari due to their higher reproductive potential and dispersal ability. I further expected surface-living Collembola species with high dispersal ability to recover more rapidly than those living deeper in the soil. Among Acari, I hypothesized Astigmata, Prostigmata and Gamasida to recover more rapidly than Oribatida due to their generally faster reproductive cycles. I expected the immediate effects of flooding to be similar in both Collembola and Acari and to be independent of plant species diversity.

In Chapter 4 I used stable isotope and fatty acid analysis to investigate intraspecific variation in the diet of two of the most abundant predatory arthropods in grasslands, the carabid beetle *Harpalus rufipes* and the wolf spider *Trochosa ruricola*. Because of its strictly carnivorous diet, I expected *T. ruricola* to occupy a higher trophic position than *H. rufipes*. Furthermore, because of its omnivory and feeding on plant seeds, I hypothesized the diet of *H. rufipes* to vary more with plant community composition than that of *T. ruricola*.

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Chapter 2

Flood-Induced Changes in Soil Microbial Functions as Modified by Plant Diversity

Odette González Macé, Katja Steinauer, Alexandre Jousset, Nico Eisenhauer,

Stefan Scheu

Flood-induced Changes in Soil Microbial Functions as Modified by Plant Diversity

Abstract

Flooding frequency is predicted to increase during the next decades, calling for a better understanding of impacts on terrestrial ecosystems and for developing strategies to mitigate potential damage. Plant diversity is expected to buffer flooding effects by providing a broad range of species' responses. Here we report on the response of soil processes to a severe summer flood in 2013, which affected major parts of central Europe. We compared soil microbial respiration, biomass, nutrient limitation and enzyme activity in a grassland biodiversity experiment in Germany before flooding, one week and three months after the flood. Microbial biomass was reduced in the severely flooded plots at high, but not at low plant functional group richness. Flooding alleviated microbial nitrogen limitation, presumably due the input of nutrient-rich sediments. Further, the activity of soil enzymes including 1,4-β-N-acetylglucosaminidase, phenol oxidase and peroxidase increased with flooding severity, suggesting increased chitin and lignin degradation as a consequence of the input of detritus in sediments. Flooding effects were enhanced at higher plant diversity, indicating that plant diversity temporarily reduces stability of soil processes during flooding. The long-term impacts, however, remain unknown and deserve further investigation.

Keywords: flooding, disturbance, climate change, nutrient limitation, microbial respiration, microbial biomass, enzyme activity, Jena Experiment.

Introduction

Climate change has increased the frequency and magnitude of extreme weather events such as wildfires and floods (Coumou & Rahmstorf, 2012; Walker, 2012). Floods affect soil structure and fertility, reducing nutrient availability and initiating primary succession processes in the case of strong disturbances (Walker, 2012). However, moderate floods also may beneficially affect nutrient cycling and increase habitat heterogeneity thereby fostering biodiversity (Walker, 2012). Biodiversity itself can influence the way ecosystems respond to disturbances such as flooding. For example, plant diversity has been shown to increase the resistance of community functions to wet (as well as dry) conditions (Isbell et al., 2015). However, results from a recent flood which hit the Jena Experiment, a large scale field experiment exploring plant diversity-ecosystem functioning relationships (Roscher et al., 2004), suggest that stability actually decreases at higher plant diversity due to greater biomass production at low flooding severity, and low biomass production at high flooding severity (Wright et al., 2015). There is little knowledge, however, about how soil processes are affected by interactions between plant diversity and disturbances like flooding (Wagner et al., 2015).

Soil microorganisms are vital for the functioning and long-term sustainability of ecosystems (Pankhurst et al., 1996). They play key roles in organic matter decomposition and nutrient cycling and thereby for plant growth. Plant diversity alters microclimatic conditions as well as the quantity and quality of litter and root resources (Eisenhauer et al., 2010; Lange et al., 2015; Spehn et al., 2000), thereby controlling the composition and functioning of soil microbial communities (Nilsson et al., 2008; Zak et al., 2003). Indeed, previous studies in the framework of the Jena Experiment suggest that microbial respiration and biomass increase with plant species richness (Eisenhauer et al., 2010; Lange et al., 2015; Strecker et al., 2016). Microorganisms at this site are limited primarily by nitrogen, with the limitation being more pronounced at low plant diversity due to lower plant N capture than under high plant diversity (Eisenhauer et al., 2010).

Floods provide rich and readily decomposable nutrients and alter nitrogen dynamics, which influence nutrient availability and alter plant growth (Hefting et al., 2004; Lockaby et al., 1996). In parallel, soil inundation also results in oxygen depletion, fostering anaerobic conditions and microorganisms able to survive these conditions (Unger et al., 2009). Such changes in soil abiotic conditions can alter soil microbial community composition (Wagner et al., 2015; Wu et al., 2015). During the flooding of the Jena Experiment, plant diversity did not attenuate flooding effects on

soil microbial communities composition (Wagner et al., 2015), even though plant community functions were altered after the flood (Wright et al., 2015).

One of the major indicators of soil microbial functioning is enzyme activity (Dick et al., 1996). Soil enzymes play an essential role in organic matter decomposition and nutrient cycling (Dick et al., 1996). Although soil enzymes mainly are synthesized by soil microorganisms, some are also produced by plant roots (Sinsabaugh, 1994). Enzymes degrade polymers of microbial and plant origin, such as cellulose, chitin and lignin, into smaller units to be used for microbial growth or plant uptake (Sanaullah et al., 2011). Enzyme activity is regulated by the demand for substrate as well as by substrate availability (Olander & Vitousek, 2000). Previous studies reported that the activity of extracellular enzymes in soil is enhanced by increasing plant diversity (Chung et al., 2007; Kreyling et al., 2008; Steinauer et al., 2015). Flooding also is likely to affect soil enzyme activities by changing nutrient availability and oxygen concentrations as well as due to shifting microbial community composition (Burns & Ryder, 2001).

In the present study we focus on changes in microbial respiration, biomass and nutrient status as well as enzyme activity associated with the 2013 summer flood which hit the Jena Experiment. We expected an immediate decrease in basal respiration, microbial biomass and enzyme activities due to anoxic conditions, particularly so at high flooding severity (Wright et al., 2015). Further, we expected effects of flooding on microbial biomass and enzyme activities to be more pronounced in higher plant diversity plots due to high oxygen consumption by roots in more diverse plant communities with higher root biomass (Mommer et al., 2010; Mueller et al., 2013; Ravenek et al., 2014). Also, we expected the flood to reduce microbial nutrient limitation due to the input of nutrient-rich sediments and the enhanced availability of dead organic material with the reduction being most pronounced at high plant diversity due to more severe nutrient limitation via more efficient plant N capture at high plant diversity. With time we expected plant diversity to foster recovery of microbial biomass and enzyme activities after the flood due to enhancing internal nutrient cycling and higher input of root-derived residues.

Material and methods

Experimental design

The experiment was located in a semi-natural temperate grassland on a eutric fluvisol (FAO-Unesco, 1997) in the floodplain of the river Saale close to the city of Jena (Thuringia, Germany, 50° 55′ N, 11° 35′ E, 130 m a.s.l.). Mean annual air temperature is 9.9°C and mean annual

precipitation is 610 mm (1980 – 2010) (Hoffmann et al., 2014). The study site had been used as arable field for over 40 years before the experiment was established in 2002. The experiment comprises 80 plots of 5 x 6 m arranged in 4 blocks to control for changes in soil texture with distance from the river. A gradient of plant species richness (1, 2, 4 8, 16 and 60) and plant functional group richness (1, 2, 3 and 4) was established comprising typical plant Central European hay meadow species. Species are grouped according to the morphological, phenological and physiological traits into grasses (16 species), small herbs (12 species), tall herbs (20 species) and legumes (12 species) (for details see Roscher et al., 2004). The established grassland is mown twice a year and weeded three times per year (Roscher et al., 2004). No permission was needed to take the samples from this site. The site is rented and managed by the project and for taking samples for analysing soil arthropods from arable systems no permission from legal bodies is needed.

Flooding event

Rainfall in May 2013 in Jena was approximatively 150 mm (>25 % of annual precipitation at the site) and the experimental field was flooded for 24 days (30 May to 24 June). Flooding caused anaerobic soil conditions, as shown by redox potentials ranging from -121 to 193 mV in the soil of some plots (Wright et al., 2015). Water coverage was measured for each plot every day from 31 May to 24 June and ascribed to 5 levels: 0, 25, 50, 75 and 100%. Flooding severity was evaluated using a continuous flooding index (sum of the percentage water coverage per day over the flooding period; Wright et al., 2015).

Microorganisms

Soil samples were taken from each plot before the flood (16th May 2013) and twice after the flood (1th July and 17th September 2013), i.e. at maximum and at late plant growth. Samples were based on composite samples of three soil cores of a diameter of 5 cm to a depth of 5 cm. The samples were homogenized, sieved (2 mm) and stored at 5°C until further analysis.

Basal respiration (BR) and substrate-induced respiration (SIR; Anderson & Domsch, 1978) were measured using an O_2 microcompensation apparatus (Scheu, 1992). Respiration was measured at 22°C with readings taken at hourly intervals. BR ($\mu L\ O_2\ g^{-1}$ soil dry weight h^{-1}) was calculated as mean O_2 consumption rates from 14 to 24 h after attachment of the samples to the respiration apparatus. SIR was measured after addition of D-glucose saturating the catabolic enzymes of the microorganisms (4 mg g^{-1} dry weight solved in 400 μL deionized water; Eisenhauer et al., 2010).

The lowest three readings within the first 10 h were averaged as the maximum initial respiratory response (MIRR; μL O_2 g^{-1} soil dry weight h^{-1}) and microbial biomass (C_{mic}; μg C g^{-1} soil dry weight) was calculated as 38 × MIRR (Beck et al., 1997).

Microbial nutrient limitations were evaluated by measuring the respiratory response after addition of D-glucose and nutrients as aqueous solution (400 μL g^{-1} soil dry weight) to fresh soil equivalent to 3.5 g dry mass. Glucose (C), glucose and nitrogen (carbon and nitrogen (CN); N as (NH_4) SO_4), glucose and phosphorus (CP; P as K_2HPO_4), and glucose, nitrogen and phosphorus (CNP) were added in a mass ratio of C: N: P of 10:2:1 (Anderson & Domsch, 1980). Respiration rates between the lowest (usually 3-6 h after substrate addition) and highest reading were taken to calculate microbial growth(Scheu, 1993). Data were ln-transformed and the slope determined by linear regression.

Enzyme activity

We measured enzymes involved in carbon, nitrogen, phosphorus and sulphur cycling. Reaction products of the lignin degrading enzymes phenol oxidase and peroxidase (C turnover) were measured spectrophotometrically using 5 mM L-3,4-dihydroxy-phenylalanine as substrate. A sample suspension was prepared from 1 g soil and 125 ml of 50 mM TRIS buffer (pH 7.9) and homogenized using an ultrasonic bath for 1 min. According to the pH value of the soil samples, pH of 7.9 was chosen for the assays. Absorbance of phenol oxidase and peroxidase was determined using 96-wells plates with four replicates of each soil sample, four blanks and six replicates of negative control (Saiya-Cork et al., 2002). The activities of phenol oxidase and peroxidase were measured at 450 nm after an incubation period of 14 h in darkness at 20°C using an absorbance microplate reader (FLUOstar Omega, BMG Labtech, Ortenberg, Germany). All soil enzyme activities were expressed in nanomoles per gram of dry soil per hour (nmol g^{-1} soil dry weight h^{-1}).

Activities of 1,4-β-N-acetylglucosaminidase, β-glucoronidase, galactosidase (all C turnover), phosphatase (P turnover), sulfatase (S turnover) and two proteases (cleaving at arginine and tyrosine residues, respectively; N turnover) were assessed with a 96-well microplate fluorogenic assay described by (Marx et al., 2001). Briefly, 0.1 g of fresh soil were dissolved in 10 ml sterile water and homogenized by shaking in a horizontal shaker at 200 min^{-1} for 30 min. The soil slurry was mixed with 40 mM of the appropriate substrate in 0.1 MES buffer (pH 6.1) and fluorescence increase recorded continuously in a Tecan M200 plate reader (ex/em 365/460, gain 60). Enzyme activity was defined as Methylumbeliferone (MUB)-equivalent released and gram of soil per

minute. In order to account for quenching fluorescence signal by soil particles, a serial dilution of MUB was set up in a reference soil slurry.

Statistical analyses

Data were inspected for normality and homoscedasticity of errors using Shapiro - Wilk normality test ($P > 0.05$) and Fligner - Killeen test ($P > 0.05$). The 60 species mixtures were excluded from the statistical analysis due to an insufficient number of replicates (four replicates at the field site, all being differentially affected by the flood; Wright et al., 2015). The difference of respective values at the two sampling dates after the flood (July or September) and before the flood (May) are used as dependent variables. Thereby, we controlled for possible confounding of flooding with other site-specific variables. Plant species richness was log-transformed to linearize the saturating relationship between plant diversity and microbial properties (Hooper et al., 2005).

Linear models were used to analyse the effects of block (categorical variable, 4 blocks), flooding index (continuous variable, from 1 to 23), log plant species richness (continuous variable, from 1 to 16), functional group richness (continuous variable, from 1 to 4) and the presence/absence of legumes, grasses, tall herbs and short herbs (categorical variable) on the differences in basal respiration, microbial biomass, microbial growth after addition of C, CP, CN and CNP and enzyme activity (phenol oxidase, peroxidase, 1,4-β-N-acetylglucosaminidase, β-glucoronidase, galactosidase, phosphatase, sulfatase, arg-protease and tyr-protease). Interactions between plant diversity (plant species richness and plant functional group richness) and the presence/absence of legumes, grasses, tall herbs and small herbs were checked against flooding index. The full model with the lowest Akaike Information Criterion (AIC) was selected as the best starting model (Faraway, 2014; Zuur et al., 2007). This model was simplified in a stepwise manner by dropping non-significant variables. Although the experimental design was set up as orthogonally as possible, there is collinearity between functional group richness of plants and the presence/absence of individual functional groups (Roscher et al., 2004), which we quantified using the inflation factor (VIF) from the car package (Fox & Weisberg, 2010). The analysis suggested to exclude functional group richness if there are two or more functional groups in the model (VIF ~ 4). Therefore, functional group richness was added after model simplification and was only included in the final model if it improved the model significantly (principle of Occam's Razor, p-value < 0.05). F-values given in text and tables refer to models in which the respective factor was fitted first (Schmid et al., 2002). Block and flooding index were fitted first followed by plant species richness; thereafter presence/absence of grasses, legumes, tall herbs and short

herbs were fitted. Statistical analyses were performed using R 3.2.1 (R Development Core Team, 2012).

<u>Results</u>

Immediate responses to the flood (July versus May)

As indicated by measurements immediately before (May) and after the flood (July) basal respiration was not significantly affected by flooding index; rather, only the presence of legumes increased basal respiration (Table 1). In contrast, microbial biomass significantly changed with flooding index, but the effect varied with plant functional group richness, with a slight increase at low plant functional group richness and a strong decrease in more severely flooded plots of high plant functional group richness (Fig. 1,Table 1). Further, differences of microbial growth of C- and CP-supplemented microorganisms increased with increasing flooding index (Fig. 2A, Table 1) and with increasing plant species richness (Fig. 3, Table 1). Moreover, legume presence significantly reduced changes in microbial growth of CP-supplemented microorganisms (Table 1). In contrast, changes in microbial growth of CN- and CNP-supplemented microorganisms did not vary significantly with flooding and plant community properties.

Differences of the activities of phenol oxidase, peroxidase and 1,4-β-N-acetylglucosaminidase were more pronounced in more heavily flooded plots (Figs. 4 AB, Table 2). Further, plant species richness significantly enhanced changes in 1,4-β-N-acetylglucosaminidase and phosphatase activity (Fig. 5A, Table 2). The presence of legumes increased changes in 1,4-β-N-acetylglucosaminidase, galactosidase and sulfatase activities, while the presence of grasses increased changes in phosphatase activities (Table 2). Small herbs presence only reduced changes in tyrosine protease activity.

Medium-term responses to the flood (September versus May)

Three months after flooding (September) changes in microbial growth of C- and CP-supplemented microorganisms were more pronounced in flooded plots (Fig. 2B, Table 1). Further, changes in microbial growth of CP-supplemented microorganisms were stronger with plant species richness and these changes were positive at highest species richness (Fig. 3). In addition, changes in microbial biomass were more pronounced in the presence of small herbs (Table 1). Moreover, changes in microbial growth of CN-supplemented microorganisms were stronger in the presence of tall herbs (Table 1).

As immediately after the flood, changes in peroxidase activity were significantly affected by flooding index in September (Fig. 4A). Further, changes in the activities of each of the enzymes measured were significantly positively affected by plant species richness except for peroxidase and phenol oxidase (Fig. 5B). Further, changes in phenol oxidase and arginine protease activity increased with the presence of grasses, while changes in arginine and tyrosine protease and galactosidase increased significantly with the presence of small herbs. Moreover, changes in phenol oxidase activity were more pronounced in presence of legumes.
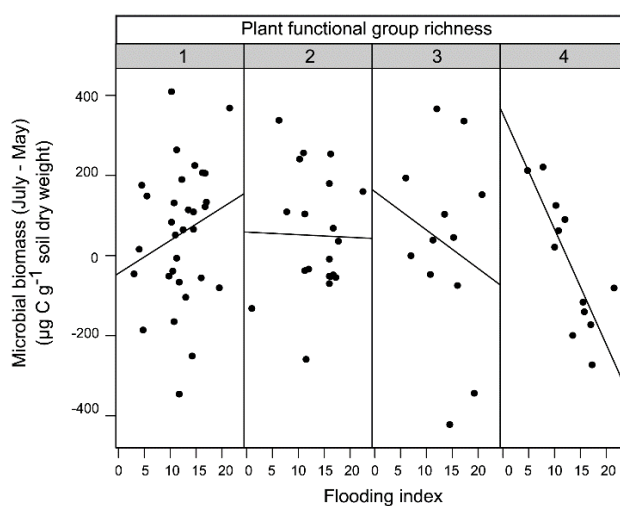

Fig. 1. Variation in microbial biomass depending on flooding index and plant functional group diversity. Effects of flooding index and plant functional groups richness (1, 2, 3 and 4) on microbial biomass changes (µg C g-1 soil dry weight) between May and July (p < 0.01). For the purposes of display only, plant functional group richness are split into 4 levels (1 to 4).
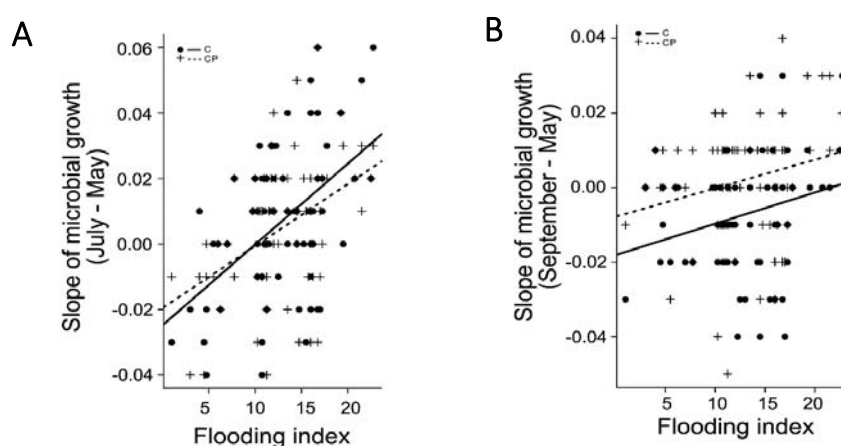

Fig. 2. Effects of flooding on C- and CP-supplemented microorganisms. Variation in slopes of regression lines of microbial growth of carbon-supplemented (C; dots) and carbon and phosphorus-supplemented (CP; cross) microorganisms between (A) May and July ($R_C^2 = 0.19$, $R_{CP}^2 = 0.20$; $p < 0.01$ for both), and (B) May and September ($R_C^2 = 0.04$, $R_{CP}^2 = 0.05$; $p < 0.05$ for both) depending on flooding index.

Table 1. Flooding and plant community effects on soil microbial functions. F-value table of linear models on the effect of block, flooding index (FI), plant functional group richness (FG), plant species richness (SR), presence of legumes (Leg), small herbs (Sh) and tall herbs (Th) on changes in basal respiration (BR), microbial biomass (C_{mic}), slope in microbial growth after addition of carbon (glucose, C), carbon and nitrogen (CN), carbon and phosphorus (CP) and carbon, nitrogen and phosphorus (CNP) between May and July, and May and September. Significant effects ($p < 0.05$) are highlighted in bold and marginally significant results ($p < 0.10$) are given in italics

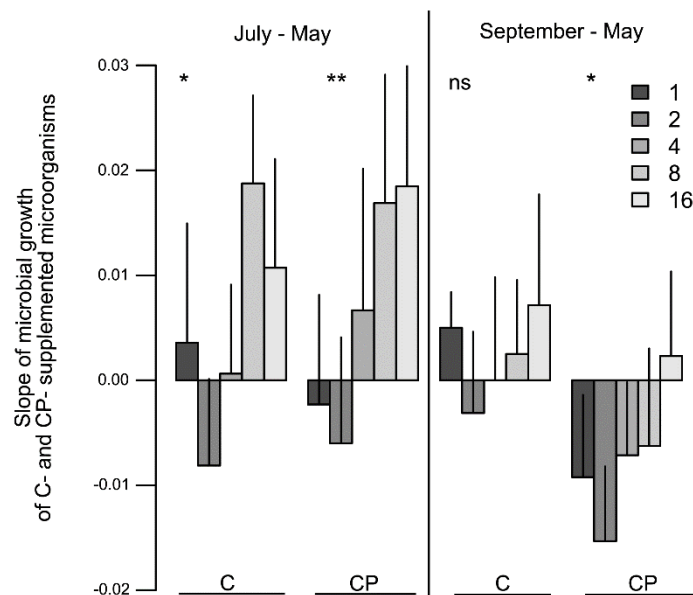|	BR		C_{mic}		C		CN		CP		CNP	
	df	F	df	F	df	F	df	F	df	F	df	F
May - July												
Block	3,51	**6.09**		-		-		-		-	3,64	*3.46*
FI		-	1,71	0.55	1,73	**18.58**		-	1,68	**23.30**		-
FG		-	1,71	1.44		-		-		-		-
SR		-		-	1,73	**6.08**		-	1,68	**8.90**		-
Leg	1,51	**10.90**		-		-		-	1,68	**12.20**		-
Interactions												
FI × FG		-	1,71	**8.50**		-		-		-		-
May - September												
Block	3,52	**6.44**		-	3,73	*3.29*		-		-	3,62	3.19
FI		-		-	1,73	**5.11**		-	1,68	**4.58**		-
SR		-		-		-		-	1,68	**4.40**		-
Sh		-	1,74	**8.13**		-	1,72	*3.67*		-		-
Th		-		-		-	1,72	**5.85**		-		-

Fig. 3. Variation in C- and CP-supplemented microorganisms depending on plant species richness. Effects of plant species richness (1, 2, 4, 8 and 16) on slope changes of microbial growth of carbon-supplemented (C) and carbon and phosphorus-supplemented (CP) microorganisms between July and May and September and May. Asterisks indicate significant differences (**$p < 0.01$, *$p < 0.05$, ns = not significant). Values are means ± SE.
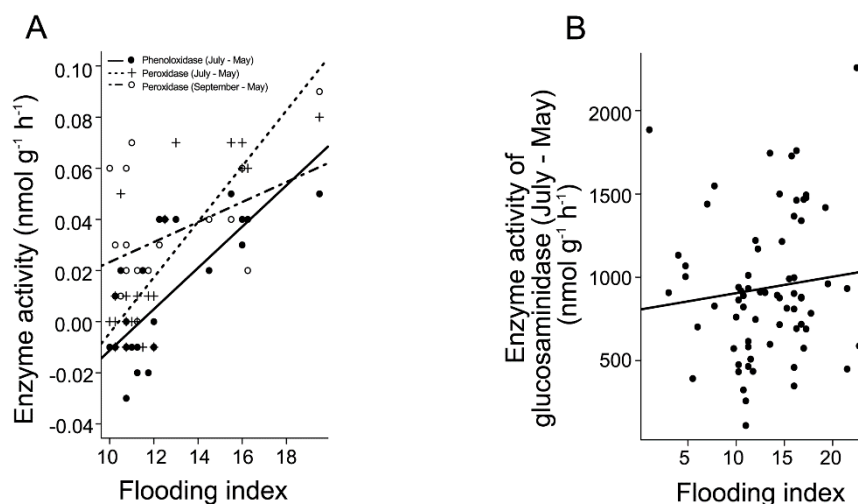
Fig. 4. Changes in enzyme activities as affected by flooding index. (A) Variation in phenol oxidase between May and July (black dots, $p < 0.01$, $R^2 = 0.58$), peroxidase between May and July (black cross, $p < 0.01$, $R^2 = 0.71$) and peroxidase between May and September (white dots, $p < 0.05$, $R^2 = 0.22$) depending on flooding index, and (B) variation in 1,4-β-N-acetylglucosaminidase activity between May and July influenced by flooding index ($p < 0.05$, $R^2 = 0.02$).
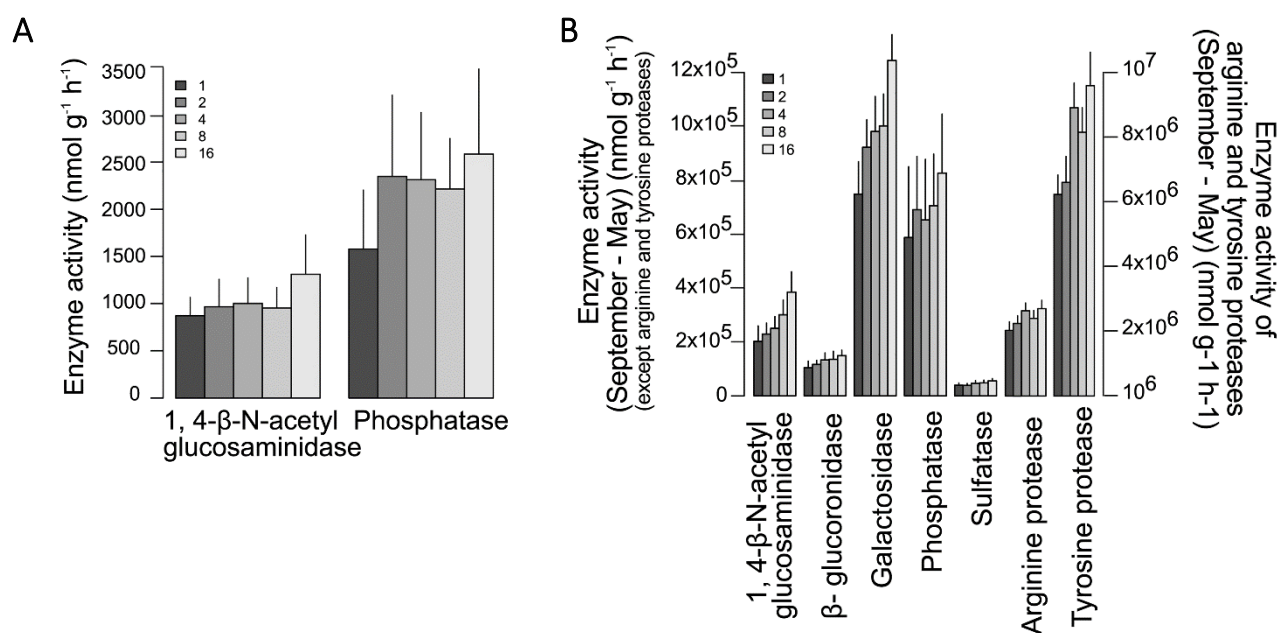
Fig. 5. Significant changes in enzyme activities as affected by plant species richness. (A) Variation in 1,4-β-N-acetylglucosaminidase and phosphatase activity between May and July affected by plant species richness (1, 2, 4, 8 and 16; $p < 0.01$), and (B) variation in glucosaminidase, β-glucoronidase, galactosidase, phosphatase, sulfatase, arginine protease and tyrosine protease activity between May and September depending on plant species richness ($p < 0.01$). Values are means ± SE.

Table 2. Flooding and plant community effects on soil enzymes. F-value table of linear models on the effect of block, flooding index (FI), plant species richness (SR), presence of grasses (Gr), legumes (Leg) and small herbs (Sh) on changes in the activity of phenol oxidase, peroxidase, 1,4-β-N-acetylglucosaminidase, β-glucoronidase, galactosidase, phosphatase, sulfatase, arginine protease and tyrosine protease between May and July, and May and September. Significant effects (P < 0.05) are highlighted in bold and marginally significant effects (P<0.10) are given in italics.

May - July

Cycle	Carbon										Phosphorus		Sulphur		Nitrogen			
Enzymes	Phenol oxidase		Peroxidase		1,4-β-N-acetyl glucosaminidase		β-glucoronidase		Galactosidase		Phosphatase		Sulfatase		Arginine protease		Tyrosin protease	
Factors	df	F	df	F	df	F	df	F	df	F	df	F	df	F	df	F	df	F
Block	1,26	**134.26**	1,24	**221.36**	3,62	**12.18**	3,65	**9.66**	3,69	**8.48**	3,66	**30.18**	3,63	**27.05**	3,71	*2.39*		-
FI	1,26	**7.01**	1,24	**23.66**	1,62	**4.47**		-		-		-		-		-		-
SR		-		-	1,62	**13.95**		-		-	1,66	**7.45**		-		-		-
Gr		-		-		-		-		-	1,66	**4.46**		-		-		-
Leg		-		-	1,62	**24.10**		-	1,69	**7.96**		-	1,63	**6.33**		-		-
Sh		-		-		-		-		-		-		-		-	1,73	**8.39**

May - September

Cycle	Carbon										Phosphorus		Sulphur		Nitrogen			
Enzymes	Phenol oxidase		Peroxidase		1,4-β-N-acetyl glucosaminidase		β-glucoronidase		Galactosidase		Phosphatase		Sulfatase		Arginine protease		Tyrosin protease	
Factors	df	F	df	F	df	F	df	F	df	F	df	F	df	F	df	F	df	F
Block		-		-	3,66	**4.80**	3,63	**7.13**	3,67	**6.10**	3,69	**92.30**	3,67	**14.99**	3,68	**4.87**	3,68	**3.91**
FI		-	1,24	**6.97**		-		-		-		-		-		-		-
SR		-		-	1,66	**26.55**	1,63	**12.93**	1,67	**42.99**	1,69	**10.74**	1,67	**15.99**	1,68	**48.37**	1,68	**14.37**
Gr	1,20	**20.86**		-		-		-		-		-		-	1,68	**4.17**		-
Leg	1,20	**5.22**		-		-		-		-		-		-		-		-
Sh		-		-		-		-	1,67	**4.32**		-		-	1,68	**5.00**	1,68	**4.69**

Discussion

Contrary to our expectation, basal respiration was not affected by flooding. This is surprising as the soils were anoxic in heavily flooded plots (Wright et al., 2015), which it is expected to infer proliferation of anaerobic microorganisms. Rather, the result suggests that aerobic microorganisms recovered quickly after flooding. As the plants also recovered fast, plant root exudation may have increased the carbon input into the soil, and, consequently, counteracted the negative effect of the flood on microbial respiration (Wu et al., 2015). Supporting this conclusion, microbial biomass also responded little to flooding, being only reduced in plots with high plant functional group diversity. Presumably, high plant functional group diversity aggravated oxygen limitation in flooded plots due to higher root biomass and root respiration as well as associated increased microbial biomass and respiration (Eisenhauer et al., 2010; Milcu et al., 2010; Reich et al., 2012; Strecker et al., 2016; Unger et al., 2009). Flooding may favor fast-growing microorganisms such as bacteria, which can readily use easily available substrates and by growing fast compensating short-term detrimental effects (Wagner et al., 2015).

As we hypothesized, limitation of microorganisms by N was reduced, likely due to an increase in soil nitrate concentrations in flooded plots. In contrast, limitation by P and C was increased after flooding with the reduction being most pronounced in plots most severely affected by flooding, likely due to dead organic material and sediments deposited by the flood (Wright et al., 2015). Of the studied enzyme activities, only those of phenol oxidase, peroxidase and 1,4-β-N-acetylglucosaminidase increased with increasing flood severity, suggesting that flooding fostered chitin and lignin degradation, presumably due to the input of additional dead organic material (Sinsabaugh, 2010; Ueno et al., 1991).

Flooding effects on soil processes were more severe at high plant diversity supporting earlier conclusions that plant diversity may compromise grassland stability (Wright et al., 2015). In high diversity plots, microorganisms are less nutrient-limited as compared to low diversity plots (Eisenhauer et al., 2010), presumably due to the input of resources of higher quality at higher plant diversity. Before the flood, microorganisms were limited primarily by the availability of N. Due to flooding, microorganisms benefited from the input of N-rich sediments in particular at high plant diversity, presumably due to complex plant structure increasing sedimentation. Moreover, mortality of some soil animals was very high during the flood (N. Eisenhauer, personal observation), which have higher densities at high plant diversity (Eisenhauer et al., 2011) and which might have also increased N availability for soil microorganisms through the turnover of

dead bodies. As a consequence, however, limitation of P and C was increased immediately after the flood.

In general, plant diversity increases both above- and belowground plant biomass (Ravenek et al., 2014; Reich et al., 2012), as well as substrate availability, microbial biomass and, as a consequence, enzyme activity (Hacker et al., 2015).The increase in the activity of phosphatase and 1,4-β-N-acetylglucosaminidase with plant species richness in July probably reflects these interrelationships. The stronger effect of plant diversity on almost all enzyme activities in September (except for peroxidase and phenol oxidase) may be related to seasonality. In September, at the end of the vegetation period when plants start to die back and increased amounts of plant residues enter the soil, microbial biomass (Habekost et al., 2008) and enzyme activity increases.

Some of the effects of flooding might have been related to characteristics of specific functional groups of microorganisms. The increase in basal respiration, 1,4-β-N-acetylglucosaminidase, galactosidase and sulfatase activity in presence of legumes shortly after the flood likely resulted from increased microbial growth due to high quality inputs (low C/N ratio) from legume litter, since legumes were most affected by severe flooding (Crème et al., 2015; Spehn et al., 2002; Wright et al., 2015). Supporting this conclusion, limitation of microorganisms by P is aggravated, whereas that by N is alleviated, in presence of legumes (Crème et al., 2015; Eisenhauer et al., 2010; Oelmann et al., 2007), and this was true in July in the present study. Three months after the flood, however, limitation by P exceeded that before the flood with the limitation being independent of the presence of legumes. The lack of legume effect in September presumably reflects that plant growth and N_2 fixation by rhizobia in the rhizosphere of legumes associated with high P demand had not recovered yet after the flood (Aerts & Chapin, 1999).

In contrast to legumes, small herbs are generally shallow-rooting with most roots being concentrated in the uppermost soil layer (Strecker et al., 2015). The presence of small herbs increased microbial biomass, galactosidase, and arginine and tyrosine protease activity three months after the flood, presumably, due to increased rhizodeposition in the topsoil. Furthermore, the presence of grasses slightly increased the activity of phosphatase in July, probably as a response to the P limitation right after the flood.

Conclusion

Flooding was associated by the input of sediments rich in nutrients. As a consequence, N limitation decreased with flooding severity. Also, the activity of enzymes degrading recalcitrant compounds such as lignin and chitin increased with flooding. Notably, these effects were more pronounced at high plant diversity and varied with plant functional group identity. Shortly after the flood, effects of legumes predominated, presumably due to high P demands of legume-associated rhizobia. In contrast, three months after the flood the effect of small herbs were most pronounced suggesting increased rhizodeposition by small herbs into the topsoil. Generally, effects of flooding were more pronounced shortly after the flood than after three months suggesting that soil microorganisms and their functioning recovered quickly resulting in nutrient limitations resembling those before the flood. Notably, even immediately after the summer flood the biomass and activity of soil microorganisms were only moderately affected despite anoxic soil conditions. Overall, the results point to high resistance and resilience of soil microbial communities in grassland with plant diversity compromising ecosystem stability. However, future studies should investigate long-term compositional changes on soil communities as well as plant community effects on soil process responses to disturbances.

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Chapter 3

Response of Collembola and Acari Communities to Summer Flooding in a Grassland Plant Diversity Experiment

Odette González Macé & Stefan Scheu

Photos from www.chaosofdelight.org

Response of Collembola and Acari communities to summer flooding in a grassland plant diversity experiment

Abstract

Flooding frequency is predicted to increase during the next decades in Europe. Therefore, it is important to understand how short-term disturbance events affect soil biota providing essential ecosystem functions and uncover factors modulating their response such as plant community composition. Here we report on the response of soil microarthropod communities (Collembola and Acari) to a severe summer flood in 2013, which affected major parts of central Europe. Collembola and Acari density and Collembola and Oribatida richness were strongly affected by the flood, but they recovered within three months. Effects of plant community composition on soil microarthropods disappeared after the flood, presumably due to homogenization of the field, but the effects of plant community were in a stage of being reasserted three months after the flood. Widespread, surface-living and generalistic microarthropod species recolonized the field quickly. Prostigmata and Oribatida were more resilient to flooding than Astigmata and Gamasida, probably taking advantage of the dead plant biomass left after the flood. Long-term impacts, however, remain unknown and deserve further investigation.

Keywords: disturbance, climate change, Oribatida, microarthropods, Jena Experiment, mesofauna.

Introduction

The increasing likelihood of extreme climate events with ongoing climate change is expected to have major impacts on biodiversity at local scales (Garcia et al., 2014). Extreme climate events will primarily consist of periods of heat, cold, drought and flooding with greater severity and less predictability than historical norms (IPCC, 2013). These events will act as disturbance and are likely to decrease biodiversity at local as well as regional scales.

Floods are projected to increase with global warming in the 21st century leading to rapid changes in soil conditions thereby detrimentally affecting soil microorganisms (Williamson & Wardle, 2007) by limiting soil gas diffusion and oxygen availability thus reducing soil nutrient availability, mineralization and decomposition of dead organic material (Schuur & Matson, 2001). As a consequence, anaerobic conditions develop quickly in flooded soils (Visser & Voesenek, 2005) resulting in marked changes in soil chemistry (Unger et al., 2010) including the accumulation of toxic substances (Schuur & Matson, 2001). All these changes are likely to significantly affect the composition of soil food webs. To explore the effects of flooding on soil food webs and, more specifically, soil microarthropods, we investigated a severe natural flooding event in a grassland plant diversity experiment (Blöschl et al., 2013). The flooding, resulting from heavy summer precipitation, was accompanied by the input of sediments rich in nutrients and was associated by an unexpectedly fast recovery of soil microorganisms within three months (González Macé et al., 2016). Fungal biomass increased, reflecting elevated availability of dead plant biomass (Wagner et al., 2015). Further, flooding was associated with enhanced plant community productivity but decreased stability, particularly of plant communities of high diversity (Wright et al., 2015).

Until today understanding how short-term disturbance events affect soil biodiversity is limited (Blankinship et al. 2011; Kardol et al. 2011) but is important as changes in soil biodiversity and community structure impact the functioning of soils (Bardgett & van der Putten, 2014; Wall et al., 2008). A major component of soil animal communities are microarthropods reaching high density and diversity in any kind of soil, and playing a crucial role in driving belowground ecosystem processes such as decomposition and nutrient cycling (Filser, 2002; Wall et al., 2008). Microarthropods, such as Acari and Collembola, are major animal groups interacting with soil microorganisms (Scheu et al., 2005; Swift et al., 1979). Microarthropod species are likely to be differentially affected by changes in environmental conditions such as inundation events depending on physiological adaptations and life history traits (Lindberg & Bengtsson 2005;

Makkonen et al. 2011). Although Collembola and Oribatida are often grouped into the same trophic level and are considered to occupy similar niches in decomposition processes (Kaneko et al. 1995; Wallwork 1970), the two groups differ in a variety of ecological traits including mobility, reproduction, level of predation pressure, and tolerance to abiotic conditions (Lindberg & Bengtsson 2005; Maraun et al. 2003; Siepel 1994). Parthenogenesis may facilitate quick population establishment after disturbances and is most widespread in Oribatida (Hoffmann et al., 2008; Norton, 1994). However, the general life-history traits of Oribatida have been considered typical of K-selected species (Norton, 1994), whereas Collembola species exhibit wider variation in life- history traits (Hopkin, 2007). In particular, compared to Collembola, Oribatida species are less mobile, characterized by low reproductive rates and recolonize disturbed habitats slowly (Maraun & Scheu, 2000). Collembola, in contrast, are more sensitive than Oribatida to abiotic microhabitat conditions and recolonize disturbed habitats more quickly (Lindberg & Bengtsson 2005; Maraun et al. 2003).

In this study, we focus on the response of Acari and Collembola to flooding in grasslands varying plant diversity. We expected the density and richness of Collembola and Acari communities to be reduced strongly by flooding with Collembola recovering faster than Acari due to higher reproductive potential and dispersal ability. We further expected that surface-living Collembola species with high dispersal ability will recover faster than species living deeper in soil (Ponge et al., 2006). Among Acari we expected Astigmata, Prostigmata and Gamasida to recover faster than Oribatida due to generally faster reproductive cycles. We further expected the immediate effects of flooding to be similar in both Collembola and Acari and to be independent of plant species diversity. However, we expected the recovery to be facilitated by high plant species diversity in particular in Collembola. Collembola density and diversity have been shown to benefit from plant diversity due to increased root and microbial biomass, and elevated quantity and quality of plant residues serving as food resources (Sabais et al. 2011; Wissuwa et al. 2013).

Material and methods

Experimental setup

The Jena Experiment is a semi-natural temperate grassland on the floodplain of the Saale river close to the city of Jena (50°55´ N, 11°35´ E; Thuringia, Germany). Mean annual air temperature is 9.9°C and mean annual precipitation is 610 mm (1980 – 2010). The study site, a eutric fluvisol, has been used as an arable field for over 40 years before the experiment was established with

typical Central European hay meadow plants in 2002. The experiment comprises 80 5 x 6 m plots arranged in 4 blocks to control for changes in soil texture with distance from the river. A gradient of plant species richness (1, 2, 4, 8, 16 and 60) and plant functional group richness (1, 2, 3 and 4) was established (Table 1). Plant species are grouped according to the morphological, phenological and physiological traits into grasses (16 species), small herbs (12 species), tall herbs (20 species) and legumes (12 species). The established grassland is mown twice a year and weeded three times per year. More details on the Jena Experiment are presented in Roscher et al. (2004).

Table 1. Design of the Jena Experiment. Combinations of plant species richness and plant functional group richness and number of replicates per diversity level. For more details on the experimental design see Roscher et al. (2004).

		Plant species diversity						
		1	2	4	8	16	60	Total
Plant	**1**	14	8	4	4	2	-	32
Functional	**2**	-	8	4	4	4	-	20
group	**3**	-	-	4	4	4	-	12
richness	**4**	-	-	4	4	4	4	16
	Total	14	16	16	16	14	4	80

Flooding event

The June 2013 flood in the Upper Danube Basin was one of the largest floods in the past two centuries (Blöschl et. al, 2013). Rainfall in May 2013 in southeast Germany was exceptionally high. In Jena it amounted to approximatively 150 mm. High rainfall resulted in the flooding of the Saale river with the flood also covering the Jena Experiment field site with the flood lasting for 24 days (30 May to 24 June). Flooding caused anaerobic soil conditions with redox potentials ranging from -121 to 193 mV in some plots (Wright et al., 2015). Water coverage was measured daily for each plot from 31 May to 24 June and ascribed to 5 levels: 0, 25, 50, 75 and 100% (percentage of the plot covered by water). Flooding severity was measured using a flooding index calculated as the sum of daily percentages for the whole flooding period (24 days), for details see Wagner et al. (2015). After the flood in August 2014, dead material, target species, weeds and bare ground percentage of the plot was measured. In general, we found that 78% of the plots was covered by target species, 14 % of weeds, 8% of dead material and 23% of bare ground. Monocultures had only 41% of target species and 50 % of bare ground. In contrast, plots with 16 plant species have 88% of the plot covered by target species and 7 % covered by bare ground. In October the vegetation was recovered totally.

Soil biota

In November 2010, July 2013 (three weeks after the flood) and in October 2013 (three months after the flood), soil cores of 5 cm diameter and 5 cm depth were taken from each plot using a stainless steel corer (80 samples per date). Soil microarthropod species were extracted using a high-gradient extractor (Macfadyen, 1961), increasing the temperature gradually from 25 to 55 °C during 14 days. The animals were collected in mono-ethyleneglycol and transferred into 70 % ethanol for preservation. Acari were sorted into Oribatida, Gamasida, Prostigmata and Astigmata. Oribatida were identified to species level using Weigmann (2006) and Collembola using Hopkin (2007) and Fjellberg (1998, 2007). For identification a light microscope (Axioplan; Zeiss, Germany) with up to ×1000 magnification was used. Density (number of individuals per square meter) and species richness (number of species) for Collembola and Oribatida were calculated.

Data analysis

To improve homogeneity of variances, data on abundance (individuals per soil core) and species richness were $\log_{10}$ (x+1) transformed prior to statistical analysis. The 60 plant species mixtures were excluded from the statistical analysis due to insufficient number of replicates (four replicates at the field site, each being differentially affected by the flood; Wright et al. 2015). Linear models (type I sum of squares) were used to analyse effects of block (categorical variable, 4 blocks), flooding index (continuous variable, from 1 to 23 days), plant functional group richness (continuous variable, from 1 to 4), plant species richness (continuous variable, from 1 to 16, log-transformed) and presence/absence of grasses, legumes, small herbs and tall herbs (categorical variables) on the density and richness of Collembola and Acari, the density of Astigmata, Gamasida, Oribatida and Prostigmata (suborders of Acari) and the density of most abundant families of Collembola (Entomobrydae, Isotomidae and Tullbergiidae) for the data of 2010 and 2013 (three months after the flood). Due to very low density three weeks after the flood these data were not analysed statistically. The full model with the lowest Akaike Information Criterion (AIC) was selected as the best starting model (Faraway 2014; Zuur et al. 2007). This model was simplified in a stepwise manner by dropping non-significant variables. Although the experimental design was set up as orthogonal as possible, there is collinearity between functional group richness of plants and the presence/absence of individual functional groups (Roscher et al., 2004), which we quantified using the inflation factor (VIF) from the car package (Fox & Weisberg, 2010). The analysis suggested to exclude functional group richness if there are two or more functional groups in the model (VIF ~ 4). Therefore, functional group richness was added after model

simplification and was only included in the final model if it improved the model significantly (principle of Occam's Razor, $p < 0.05$). F-values given in text and tables refer to models in which the respective factor was fitted first (Schmid et al., 2002). Generally, block and flooding index were fitted first followed by plant species richness; thereafter presence/absence of grasses, legumes, tall herbs and short herbs were fitted. Statistical analyses were performed using R 3.2.1 (R, 2014).

Data on Collembola species were analysed using non-metric multidimensional scaling (NMDS with Bray-Curtis distance) reducing the number of dimensions to four. To identify the factors which drive Collembola community composition, the four dimensions were further analysed by MANOVA. In addition, discriminant function analysis (DFA) was carried out on four NMDS axes with Statistica 13 (Statsoft, Inc., Tulsa, Oklahoma, USA). Plant species and plant functional group richness were used as variables of discrimination. Squared Mahalanobis distances between groups were calculated to identify differences between plant richness levels.

The community structure of Collembola and Oribatida was analysed using principal component analysis (PCA) as implemented in CANOCO 5 (Microcomputer Power, Ithaca, NY; Ter Braak & Smilauer, 2012) using the abundance of species which appeared more than in three samples. Moreover, we correlate the factors and the axes of each PCA using Pearson correlation.

<u>Results</u>

Collembola

In November 2010 Collembola density was 22,310 ± 15,975 individuals m^{-2}, whereas three weeks after the flood in 2013 it was only 515 ± 1347 individuals m^{-2}. In contrast, three months after the flood Collembola density (23,220 ± 17,826 individuals m^{-2}) was similar to the level in 2010 (Fig. 1A,C). Collembola density in 2010 was not influenced by experimental treatments, but three months after the flood in 2013 it increased slightly with plant species richness ($F_{1,76} = 2.97$; Table 2).

In 2010 a total of 27 species of Collembola were recorded, while only 16 species were recorded three weeks after the flood. However, three months after the flood species number increased to 22 (see Supplementary Table S1). In 2010 Collembola species richness increased marginally significant with plant species richness ($F_{1,76} = 3.76$; Fig. 2A) and plant functional group richness ($F_{1,76} = 2.82$; Table 2). There was no significant effect of plant species and plant functional

group richness on Collembola species richness three months after the flood in 2013, but increased significantly with the presence of tall herbs (Table 2).

In 2010 the density of the most abundant family of Collembola, Isotomidae, increased significantly with plant species richness (Fig. 3A) and also in presence of grasses, but decreased in the presence of tall herbs. In contrast, the densities of Entomobryidae and Tullbergiidae were not significantly affected by experimental treatments in 2010 (Table 3). Three months after the flood in 2013 the density of Entomobryidae increased with flooding index ($F_{1,73}$ = 3.88). Further, the density of Tullbergiidae increased significantly with plant species richness (Fig. 3B) and decreased slightly with the presence of grasses ($F_{1,73}$ = 3.28; Table 3). In contrast to 2010, Isotomidae were not significantly affected by experimental treatments in 2013.

In 2010, Collembola community composition changed significantly with plant species richness ($F_{1,76}$= 5.33, P < 0.01) and plant functional group richness ($F_{1,76}$= 4.35, P < 0.01). Collembola community (number of species and species composition) was similar at higher plant species richness but less variable in the one and two species treatments (Table 4A). Similarly, community composition of Collembola differed between plant functional group one and four as well as two and four (Table 4B).

PCA separated Collembola communities mainly along the first axis representing 25.28 % of the variability in species data, whereas the second axis represented 15.96 % of the variability (Fig. 4). Separation along the first axis mainly represents differences between Collembola communities in 2010 and three months after the flood in 2013 (r = - 0.75). The most abundant species before the flood compared to 2013 were *Parisotoma notabilis, Mesaphorura macrochaeta, Ceratophysella denticulata* and *Onychiurus jubilarius*. After the flood the most abundant species compared to 2010 were *Lepidocyrtus lanuginosus* and *Cryptopygus thermophilus*. The second axis represents differences between plant species richness (r = 0.22), plant functional group richness (r = 0.13) and presence/absence of small herbs (r = 0.16). In general, at higher plant species richness *Lepidocyrtus cyaneus* and *Stenaphorura denisi* were more abundant. Moreover, there were some species present at each of the sampling dates including *Lepidocyrtus lanuginosus, Lepidocyrtus cyaneus* and *Willowsia buski* (Entomobryidae) as well as *Isotoma viridis, Parisotoma notabilis* and *Isotomiella minor* (Isotomidae). Other species like *Hypogastrura manubrialis, Ceratophysella engadinensis* (Hypogastruridae), *Isotomurus fucicolus, Proisotoma minuta* (Isotomidae)*, Paratullbergia macdougalli* (Tullbergiidae), *Protaphorura armata* (Onychiuridae) and *Sminthurus viridis* (Sminthuridae) were present only three weeks after the flood.

Acari

In November 2010 Acari density was 21,500 ± 23,290 individuals m^{-2}, but only 1,864 ± 3,059 individuals m^{-2} three weeks after the flood in 2013. In contrast, similar to Collembola, three months after the flood in 2013 (27,350 ± 30,040 individuals m^{-2}) it was similar to the level in 2010 (Fig. 1A). In 2010 and three months after the flood in 2013, Acari density increased significantly with the presence of grasses. Moreover, three months after the flood in 2013 it was significantly higher with the presence of small herbs (Table 2).

The density of each of the suborders of Acari (Oribatida, Gamasida, Astigmata, Prostigmata) decreased significantly three weeks after the flood in 2013. In contrast, three months after the flood in 2013 the density of each of the Acari suborders reached a similar level than in 2010, except of Prostigmata which exceeded the density in 2010 by more than a factor of two (Fig. 1B).

A total of 12 species of Oribatida were recorded in 2010, but only 9 species were recorded three weeks after the flood in 2013. However, similar to Collembola, Oribatida species also recovered quickly with 12 species being present three months after the flood in 2013 (see Supplementary Table S2). In 2010 Oribatida richness increased significantly with plant species richness (Fig. 2B) and plant functional group richness. Further, in 2010 ($F_{1,76} = 2.94$) as well as three months after the flood in 2013 ($F_{1,76} = 3.04$) Oribatida richness increased with the presence of grasses, however, only slightly (Table 2). Three months after the flood in 2013 Oribatida richness was slightly reduced at higher flooding index ($F_{1,76} = 2.86$; Table 2).

Oribatida density increased significantly with plant species richness both in 2010 and three months after the flood in 2013 (Fig. 5A,B). Moreover, in 2010 Oribatida density increased significantly with plant functional group richness and the presence of grasses (Table 5). In 2010 Gamasida density decreased slightly with the presence of tall herbs ($F_{1,70} = 3.58$), while in 2013 it increased significantly with plant species richness (Fig. 5D) and plant functional group richness as well as the presence of legumes and slightly small herbs ($F_{1,70} = 3.01$; Table 5). Prostigmata density increased slightly with plant functional group richness ($F_{1,70} = 3.75$) and significantly in presence of grasses but only in 2010. In 2013, it was not significantly affected by experimental treatments (Table 5). Astigmata density was significantly higher at higher plant species richness (Fig. 5C) and in presence of grasses in 2010 ($F_{1,70} = 3.59$) but not in 2013 (Table 5).

PCA separated Oribatida species along the first axis explaining 48.2 % of the variability in species data and the second axis representing 15.99 % of the variability in species data (Fig. 6).

Separation along the first axis mostly represents differences between Oribatida communities in 2010 and three months after the flood in 2013 (r = 0.11) and the presence of legumes (r = 0.13) and grasses (r = 0.15). The second axis mainly represents differences with plant functional group richness (r = 0.14) and presence of grasses (r = 0.19). Separation along the first axis were due to e.g., *Oppiella nova* being more abundant three months after the flood in 2013. Separation along the second axis was due to e.g., higher numbers of *Oribatula excavata* in plant communities with grasses. Moreover, we found some species present at each of the sampling including *Oppiella nova, Tectocepheus sarekensis, Oribatula excavata, Rhysotritia ardua* and *Punctoribates punctum* as well as species present only three weeks after the flood like *Zygoribatula frisiae* and *Schleloribates initialis.*
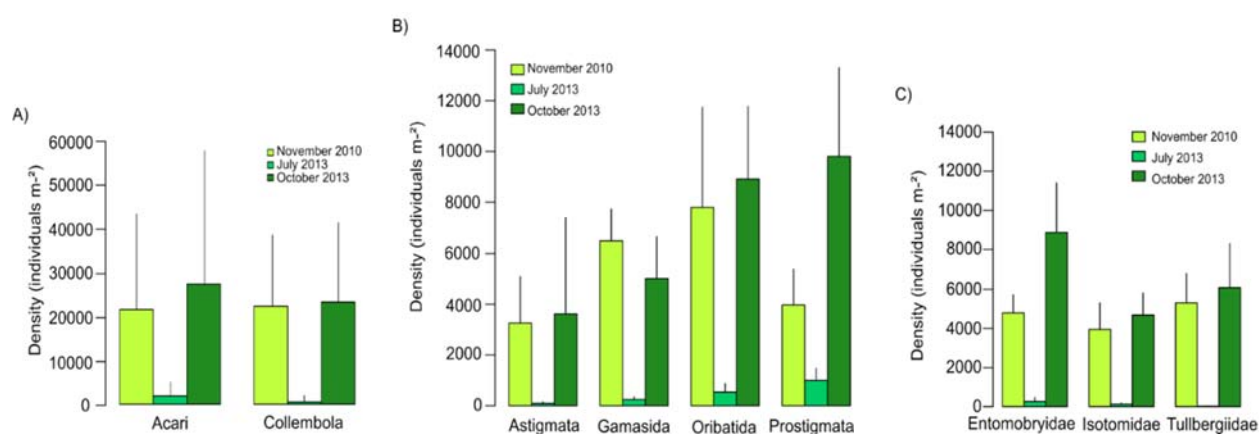

Fig. 1. Density of Acari and Collembola. Density of (A) Acari and Collembola, (B) Acari suborders (Astigmata, Gamasida, Oribatida and Prostigmata) and (C) Collembola families (Entomobryidae, Isotomidae, Tullbergiidae) in November 2010 (before the flood, 80 samples), July 2013 (three weeks after the flood, 80 samples) and October 2013 (three months after the flood, 80 samples). Values are means ± SE.
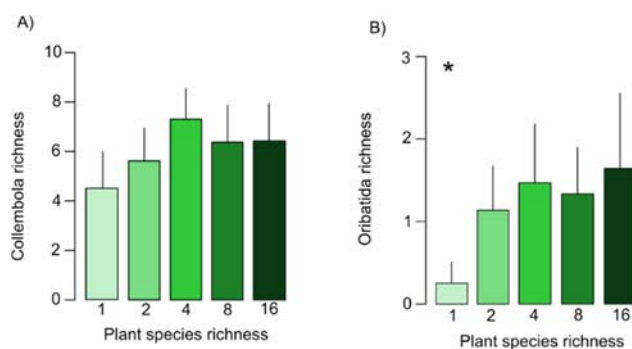

Fig. 2. Effect of plant species richness on species richness of Collembola and Oribatida. Variations in species richness of (A) Collembola and (B) Oribatida with plant species richness (1, 2, 4, 8, 16 species with 14, 16, 16, 16 and 14 replicates, respectively) in November 2010. Values are means ± SE. For statistical analysis see Table 2.
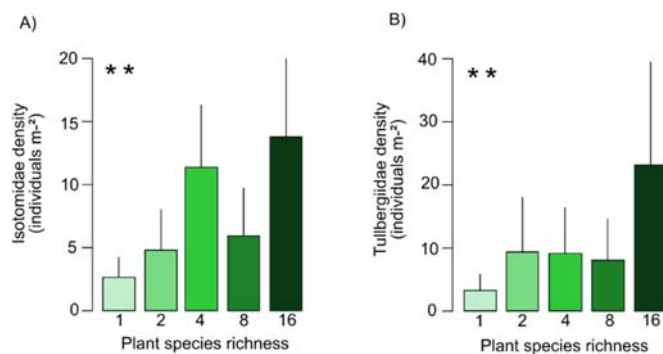
Fig. 3. Effects of plant species richness on the density of Isotomidae and Tullbergiidae. Variations in density of (A) Isotomidae in November 2010 and (B) Tullbergiidae in October 2013 with plant species richness (1, 2, 4, 8, 16 with 14, 16, 16, 16 and 14 replicates, respectively). Values are means ± SE. For statistical analysis see Table 3.
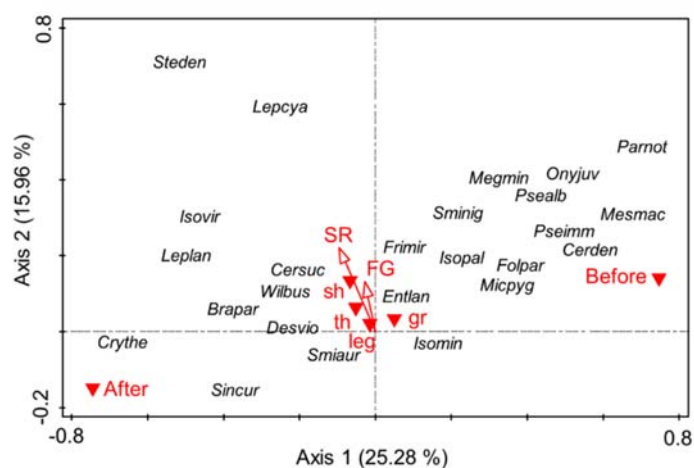

Fig. 4. PCA of Collembola species. Principal components analysis (PCA) of Collembola species in plant communities in November 2010 (before the flood) and October 2013 (three months after flood). Arrows indicate variations with plant species (SR) and plant functional group diversity (FG). Variations with the presence of grasses (gr), legumes (leg), small (sh) and tall herbs (th) and between November 2010 (before) and October 2013 (after) are indicated by red triangles. For full species names see Table S1.
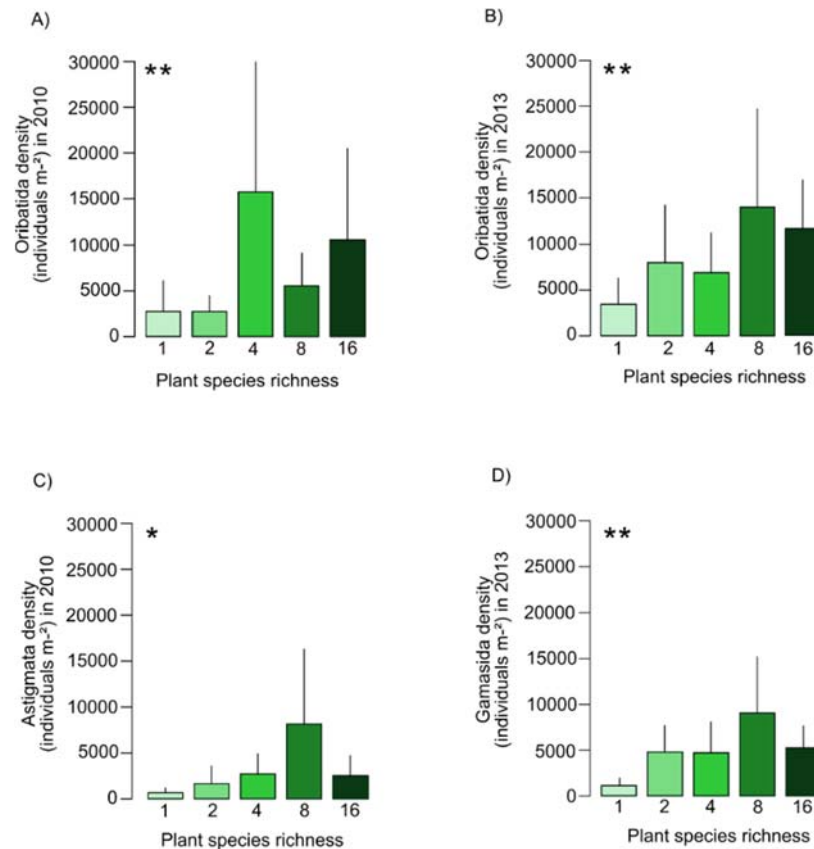
Fig. 5. Effects of plant species diversity on the density of Oribatida, Astigmata and Gamasida. Variation in density of (A) Oribatida in November 2010, (B) Oribatida in October 2013 (three months after the flood), (C) Astigmata in November 2010, and (D) Gamasida in October 2013 with plant species richness (1, 2, 4, 8, 16 with 14, 16, 16, 16 and 14 replicates, respectively). Values are means ± SE. For statistical analysis see Table 5.
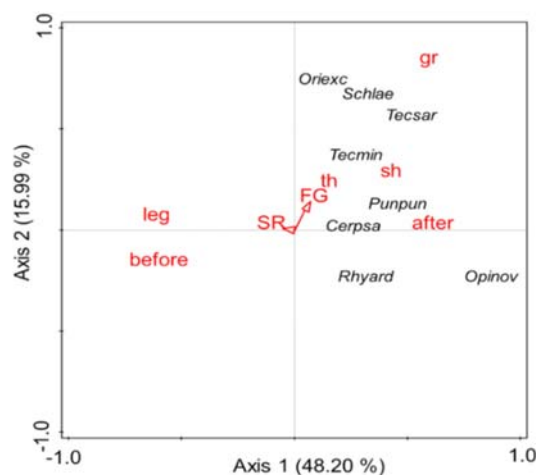

Fig. 6. PCA of Oribatida species. Principal components analysis (PCA) of Oribatida species in plant communities in November 2010 (before the flood) and October 2013 (three months after flood). Arrows indicate variations with plant species (SR) and plant functional group diversity (FG). Variations with the presence of grasses (gr), legumes (leg), small (sh) and tall herbs (th) and between November 2010 (before) and October 2013 (after) are indicated by red triangles. For full species names see Table S2.

Table 2. Table of F-values of linear models on the effect of block, flooding index (FI), plant species richness (SR), plant functional group richness (FG), presence of grasses (gr), small herbs (sh) and tall herbs (th) on the density and richness of Acari and Collembola in November 2010 (before the flood) and October 2013 (three months after the flood). Significant effects are given in bold (P < 0.05) and marginally significant effects in italics (P<0.10)

	Acari								Collembola							
	Acari density				Oribatida richness				Collembola density				Collembola richness			
Year	2010		2013		2010		2013		2010		2013		2010		2013	
	df	F	df	F	df	F	df	F	df	F	df	F	df	F	df	F
Block	3,76	**4.83**		-	1,76	**9.76**		-		-		-		-		-
FI		-		-		-	1,76	↓*2,86*		-		-		-		-
SR		-		-	1,76	↑**6.30**		-		-	1,76	↑*2.97*	1,76	↑*3.76*		-
FG		-		-	1,76	↑**5.18**		-		-		-	1,76	↑*2.82*		-
gr	1,76	↑**6.19**	1,76	↑**5.27**	1,76	↑*2.94*	1,76	↑*3,04*		-		-		-		-
sh		-	1,76	↑**4.06**		-		-		-		-		-		-
th		-		-		-		-		-		-		-	1,76	↑**4.54**

Table 3. Table of F-values of linear models on the effect of block, flooding index (FI), plant species richness (SR), presence of grasses (gr) and tall herbs (th) on the density of Entomobryidae, Isotomidae and Tullbergiidae in November 2010 (before the flood) and October 2013 (three months after the flood). Significant effects (P < 0.05) are given in bold and marginally significant effects (P<0.10) in italics.

	Entomobryidae				Isotomidae				Tullbergiidae			
Year	2010		2013		2010		2013		2010		2013	
	df	F	df	F	df	F	df	F	df	F	df	F
Block	-		-		-		-		-		3,73	**3.11**
FI	-		1,73	↑*3.88*	-		-		-		-	
SR	-		-		1,73	↑**7.18**	-		-		1,73	↑**9.57**
gr	-		-		1,73	↑**4.53**	-		-		1,73	↓*3.28*
th	-		-		1,73	↓**4.65**	-		-		-	

Table 4. Squared Mahalanobis distances between group centroids and reliability of discrimination for Collembola species composition in November 2010 of (A) plant species richness, (B) plant functional group richness; **P < 0.05, *P < 0.10.

A)

Plant species richness	1	2	4	8	16
1	-	1.95*	1.72	2.45**	1.47
2		-	2.56**	1.59*	2.43**
4			-	0.68	0.88
8				-	0.92
16					-

B)

Plant fuctional group	1	2	3	4
1	-	0.82	1.02	1.68**
2		-	0.97	1.41*
3			-	0.50
4				-

Table 5. Table of F-values of linear models on the effect of block, flooding index (FI), plant species richness (SR), plant functional group richness (FG), presence of grasses (gr), legumes (leg), small herbs (sh) and tall herbs (th) on the density of Astigmata, Gamasida, Oribatida and Prostigmata in November 2010 (before the flood) and October 2013 (three months after the flood). Significant effects are given in bold (P < 0.05) and marginally significant effects in italics (P<0.10).

	Astigmata				Gamasida				Oribatida				Prostigmata			
Year	2010		2013		2010		2013		2010		2013		2010		2013	
	df	F	df	F	df	F	df	F	df	F	df	F	df	F	df	F
Block	-	-	-	-	3,70	**3.64**	-	-	3,70	**6.42**	-	-	3,70	**6.97**	-	-
SR	1,70	↑**6.24**	-	-	-	-	1,76	↑**13.18**	1,70	↑**8.77**	1,76	↑**10.46**	-	-	-	-
FG	-	-	-	-	-	-	1,76	↑**16.74**	1,70	↑**6.28**	-	-	1,70	↑*3.75*	-	-
gr	1,70	↑*3.59*	-	-	-	-	-	-	1,70	↑**4.49**	-	-	1,70	↑**5.17**	-	-
leg	-	-	-	-	-	-	1,76	↑**3.99**	-	-	-	-	-	-	-	-
sh	-	-	-	-	-	-	1,76	↑*3.01*	-	-	-	-	-	-	-	-
th	-	-	-	-	1,70	↓*3.58*	-	-	-	-	-	-	-	-	-	-

Discussion

Collembola and Acari density as well as Collembola and Oribatida species richness were affected drastically by the flood but recovered quickly, returning within three months to levels recorded three years earlier (2010), despite of the seasonal changes of the community, since generally the Collembola and Acari abundance in autumn is higher than in summer (Sabais et al., 2011; Fig. S1). This reinforces assumptions that microarthropods respond rapidly to environmental changes (Chauvat et al., 2003) and recover quickly (Lindberg & Bengtsson 2006). In 2010 Collembola and Oribatida species richness increased with plant species and plant functional group richness, but these interrelationships were absent three months after the flood. This suggests that flooding resulted in homogenization of environmental conditions, eradicating effects of plant community composition established before the flood. However, three months after the flood Collembola species richness was increased in the presence of tall herbs and Oribatida density and richness were consistently affected by grasses, indicating that effects of plant community composition were in a stage of being reasserted.

As hypothesized, Entomobryidae, as epedaphic species, may benefit from their dispersal ability thereby quickly recovering after the flood, presumably taking advantage of increased fungal biomass in the most severely flooded plots (Wagner et al., 2015). In contrast to Entomobryidae, the euedaphic and hemiedaphic Tullbergiidae and Isotomidae require habitable pore space (Bardgett, 2005) and this likely contributed to the delayed recolonization of the clogged flooded soils. They also are more reliant on the recovery of the plant community. Isotomidae and Tullbergiidae are sensitive to soil quality and root exudates (Milcu et al., 2010), and are assumed to benefit from increased root biomass and associated exudates in more diverse plant communities (Ravenek et al., 2014). In 2010 the density of Isotomidae increased in presence of grasses and tall herbs. Grasses increase root and microbial biomass, both likely contributing to increased food resource supply to Collembola (Endlweber & Scheu, 2007; Sabais et al., 2011; Salamon et al., 2004).

Oribatid mites are decomposers and have been used as indicators of soil stability and fertility (Socarrás & Izquierdo, 2014). Generally, they have low metabolic rates, slow development and low fecundity. Consequently, they are vulnerable to disturbances (Lindberg, 2003) and recover slowly thereafter (Behan-Pelletier 1999; Scheu & Schulz, 1996). However, higher densities of Prostigmata and Oribatida than of Astigmata and Gamasida three weeks after the flood suggest that the former two taxa are more resistant to flooding that the latter. Detrimental effects of

flooding may have been alleviated by dead plant biomass functioning as shelter for Prostigmata and Oribatida (Wright et al., 2015). Notably, three months after the flood, the density of Prostigmata was twice that in 2010. Prostigmata are dominant Acari predators with a crucial role in soil food webs important as biological control agents (Walter & Proctor, 1999). Some species are known to benefit from habitat disturbance (Wallwork, 1980). Prostigmata, Gamasida and Astigmata are assumed to be r-strategists frequently occurring in disturbed habitats (Behan-Pelletier, 2003) and quickly colonizing new habitats due to high dispersal ability, high fecundity and fast development (Norton, 1994). However, in contrast to Prostigmata, Gamasida and Astigmata did not take advantage of the flood. The majority of Gamasida are mobile predators feeding on Nematoda, Collembola, Enchytraeidae, larvae of Insecta and Acari (Koehler, 1999). Conform to their assumed sensitivity to environmental changes (Ruf & Beck, 2005), their density increased with plant species and plant functional group richness as well as in presence of legumes three months after the flood in 2013. This likely resulted from increased availability of prey such as Collembola (Isotomidae) and nematodes both increasing significantly with plant diversity three months after the flood (Wagner et al., 2015).

As ecosystems develop after disturbances, changes in soil are likely to be associated by corresponding changes in the soil community (Bokhorst et al., 2017; Kaufmann et al., 2002). As indicated by the dramatic decline in the density and species richness of Collembola and Acari, flooding represented a strong disturbance for soil microarthropods. The community composition of Collembola markedly changed after the flood and this lasted for at least three months. On one hand some species were present at each of the sampling dates, including *Lepidocyrtus lanuginosus, Lepidocyrtus cyaneus* and *Willowsia buski* (Entomobryidae) as well as *Isotoma viridis, Parisotoma notabilis* and *Isotomiella minor* (Isotomidae) (see Supplementary Table S1). Each of these species had been recorded three weeks after the flood suggesting that they survived the flooding in some of the plots and then recovered quickly confirming their generalistic lifestyle (Hopkin, 2007). On the other hand, *Hypogastrura manubrialis, Ceratophysella engadinensis* (Hypogastruridae), *Isotomurus fucicolus, Proisotoma minuta* (Isotomidae)*, Paratullbergia macdougalli* (Tullbergiidae), *Protaphorura armata* (Onychiuridae) and *Sminthurus viridis* (Sminthuridae) were present three weeks after the flood (as single individuals or in few numbers only) but neither three months after the flood nor in 2010 before the flood (see Supplementary Table S1) suggesting that a number of Collembola species were introduced by the flood. In contrast, *Zygoribatula frisiae* and *Schleloribates initialis* of Oribatida were only present three weeks after the flood. Furthermore, *Oppiella nova, Tectocepheus sarekensis*, *Oribatula excavata*, *Rhysotritia ardua* and *Punctoribates punctum* were present in all the dates and most of them

quickly increased in density suggesting they are resistant against disturbances and respond in an generalistic way. In fact, these species are known as cosmopolitan generalistic species present in any kind of habitat (Walter & Proctor 1999).

Aknowledgments

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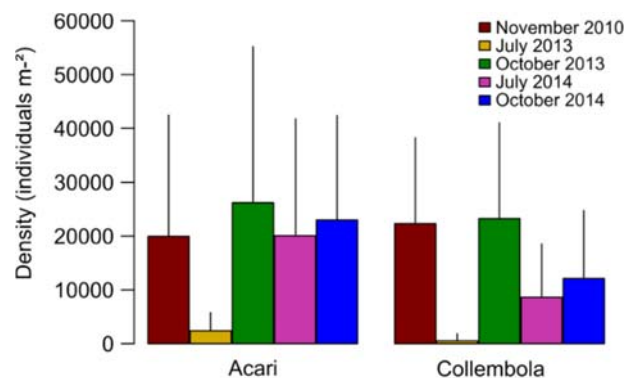
Supplementary material

Fig. S1. Density of Collembola and Acari (individuals m^{-2}) in different years (November 2010, July 2013, October 2013, July 2014 and October 2014).

Table S1. List of Collembola species, their abbreviations used in Fig. 4.and their total numbers at each of the three sampling dates.

Family	Species	Abbrev.	Nov. 2010	July 2013	Oct. 2013
Brachystomellidae	*Brachystomella parvula* (Schaeffer, 1896)	Brapar	4	-	19
Entomobryidae	*Entomobrya lanuginosa* (Nicolet, 1842)	Entlan	5	-	-
	Entomobrya multifasciata (Tullberg, 1871)	Entmul	2	-	-
	Heteromurus nitidus (Templeton, 1835)	Hetnit	1	-	1
	Lepidocyrtus cyaneus (Tullberg, 1871)	Lepcya	579	27	1,216
	Lepidocyrtus lanuginosus (Gmelin, 1790)	Leplan	13	6	102
	Pseudosinella alba (Packard, 1873)	Psealb	73	-	5
	Pseudosinella immaculata (Lie-pettersen, 1897)	Pseimm	78	-	-
	Sinella curviseta (Brook, 1882)	Sincur	-	-	59
	Willowsia buski (Lubbock, 1870)	Wilbus	2	6	12
Hypogastruridae	*Ceratophysella denticulata* (Bagnall, 1941)	Cerden	295		22
	Ceratophysella engadinensis (Gisin, 1949)	Cereng	-	1	-
	Ceratophysella succinea (Gisin, 1949)	Cersuc	-	-	9
	Hypogastrura manubrialis (Tullberg, 1869)	Hypman	-	1	-
Isotomidae	*Cryptopygus thermophilus* (Axelson, 1900)	Crythe	-	-	364
	Desoria violacea (Tullberg, 1876)	Desvio	-	-	3
	Folsomia quadrioculata (Tullberg, 1871)	Folqua	-	2	1
	Folsomides parvulus (Stach, 1922)	Folpar	47	-	-
	Isotoma viridis (Bourlet, 1839)	Isovir	57	11	262
	Isotomiella minor (Schaeffer, 1896)	Isomin	49	1	77
	Isotomodes productus (Axelson, 1906)	Isopro	-	-	18
	Isotomurus fucicolus (Reuter, 1891)	Isofuc	-	1	-
	Isotomurus palustris (Müller, 1776)	Isopal	67	-	-
	Parisotoma notabilis (Schaeffer, 1896)	Parnot	400	3	10
	Proisotoma minuta (Tullberg, 1871)	Promin	-	1	-
Katiannidae	*Sminthurinus aureus* (Lubbock, 1862)	Smiaur	7	-	18
	Sminthurinus elegans (Fitch, 1862)	Smiele	1	-	-
	Sminthurinus niger (Lubbock, 1868)	Sminig	45	1	-
Neanuridae	*Friesea mirabilis* (Tullberg, 1871)	Frimir	10	-	1
	Micranurida pygmaea (Börner, 1901)	Micpyg	32	-	16

Family	Species	Abbrev.	Nov. 2010	July 2013	Oct. 2013
Neelidae	*Megalothorax minimus* (Willem, 1900)	Megmin	92	-	2
Onychiuridae	*Onychiurus jubilarius* (Gisin, 1957)	Onyjub	162	-	-
	Protaphorura armata (Tullberg, 1869)	Proarm	-	4	-
	Supraphorura furcifera (Börner, 1901)	Supfur	3	-	-
Sminthuridae	*Sminthurus viridis* (Linnaeus, 1758)	Smivir	-	1	-
Sminthurididae	*Sphaeridia pumilis* (Krausbauer, 1898)	Spapum	1	1	-
Tullbergiidae	*Mesaphorura macrochaeta* (Rusek, 1976)	Mesmac	498	-	13
	Metaphorura affinis (Börner, 1903)	Metaff	47	-	-
	Paratullbergia macdougalli (Bagnall, 1936)	Parmac	-	3	-
	Stenaphorurella denisi (Bagnall, 1935)	Steden	288	-	943

Table S2. List of Oribatida species, their abbreviations used in Fig. 6.and their total numbers at each of the three sampling dates.

Family	Species	Abbrev.	Nov. 2010	July 2013	Oct. 2013
Ceratozetidae	*Ceratozetes psammophilus* (Horak, 2000)	Cerpsa	3	-	10
Euphthiracaridae	*Rhysotritia ardua ardua* (Koch, 1841)	Rhyard	39	1	15
Hypochthoniidae	*Hypochthonius rufulus* (Koch, 1835)	Hypruf	-	-	1
Liebstadiidae	*Liebstadia similis* (Michael, 1888)	Liesim	21	-	-
Nothridae	*Nothrus anauniensis* (Canestrini & Fanzago,	Notana	2	-	-
	Nothrus pratensis (Sellnick, 1928)	Notpra	6	-	-
Oppiidae	*Microppia minus* (Paoli, 1908)	Micmin	-	10	1
	Oppiella nova (Oudemans, 1902)	Opinov	165	1	329
Oribatulidae	*Oribatula excavata* (Berlese, 1916)	Oriexc	246	5	42
	Zygoribatula frisiae (Oudemans, 1900)	Zygfri	-	2	-
Phthiracaridae	*Steganacarus striculus* (Koch, 1836)	Stestr	-	-	1
Protoribatidae	*Protoribates capucinus* (Berlese, 1908)	Procap	-	-	1
Punctoribatidae	*Punctoribates punctum* (Koch, 1839)	Punpun	20	4	28
Scheloribatidae	*Scheloribates (Hemileius) initialis* (Berlese,	Schini	-	20	-
	Scheloribates laevigatus (Koch, 1835)	Schlae	9	12	74
Tectocepheidae	*Tectocepheus minor* (Berlese, 1903)	Tecmin	2	-	4
	Tectocepheus velatus sarekensis (Trägårdh,	Tecsar	64	30	61
	Tectocepheus velatus velatus (Michael, 1880)	Tecala	1	-	-

Chapter 4

Variations in Trophic Niches of Generalist Predators with Plant Community Composition As Indicated by Fatty Acids and Stable Isotopes

Odette González Macé, Anne Ebeling, Nico Eisenhauer, Simone Cesarz, Stefan Scheu

Photos from Roy Anderson (left) and Паук-мизгирь (right).

Variations in Trophic Niches of Generalist Predators with Plant Community Composition As Indicated by Fatty Acids and Stable Isotopes

Abstract

Arthropods are a dominant component of biodiversity in terrestrial ecosystems. They are considered pest control agents and drive important ecosystem processes like nutrient cycling. However, such ecosystem effects of arthropods may depend on the environmental context influencing nutrition and behaviour. In the framework of a grassland plant diversity experiment, we used stable isotope and fatty acid analysis to investigate intraspecific variations in the diet of two of the most abundant predatory arthropods in grasslands: the ground beetle *Harpalus rufipes* and the wolf spider *Trochosa ruricola*. The results show that the diet of *H. rufipes* varied significantly with plant species diversity, consuming more plant material, probably seeds, at higher diversity plots. In the presence of grasses and small herbs *H. rufipes* diet had an increase of seeds. Moreoever, *H. rufipes*' diet changed with the presence of legumes, increasing the proportion of animal prey, like aphids or collembolans. In contrast, the diet of *T. ruricola* consisted of animal prey only and varied mainly with body size, with larger individuals occupying a higher trophic position in the food web. Moreover, the diet of *T. ruricola* changed in response to a summer flood, which occurred two months after the samples were taken. Presumably this changed was caused due to increased availability of secondary decomposers as well as elevated intraguild predation in severely flooded plots. As both species are considered pest control agents, these results underline the importance of high local plant diversity and the composition of grassland communities for natural pest control.

Keywords: carabid bettle, spider, *Trochosa ruricola*, *Harpalus rufipes*, flood, plant diversity.

Introduction

Knowledge of the diet of consumers is essential for understanding ecological and trophic interactions, such as niche relationships, competition, coexistence and predation (Cantor et al., 2010; García et al., 2009; Vieira & Port, 2007). In particular for improving biological pests control, knowledge of predator-prey interrelationships is essential (Kromp, 1999; Wilby & Thomas, 2002). This applies to the level of species but also of individuals as individual variation in the diet is a significant component of niche variation (Bolnick et al., 2003; Roeder & Behmer, 2014; Sih et al., 2004). Thus, intraspecific variation and plasticity may provide a key mechanism promoting species coexistence (Clark et al., 2007) and plays an important role in intra- and interspecific interactions that shape population and community dynamics (Agrawal et al., 2007; McGill et al., 2006).

From a community perspective, generalist predators are important control agents of lower trophic level consumers such as herbivores (Romero & Harwood, 2010; Wise, 1993). In terrestrial ecosystems, spiders and predatory beetles are among the most important antagonists of insect herbivore species and play a major role in insect pest control (Kulkarni et al., 2015; Symondson et al., 2002). However, spiders and carabid beetles typically occupy different niches and complement each other in prey population control. Carabids are considered opportunistic generalist predators (Gallandt et al.,2005; Lee et al., 2001), with some of them consuming substantial amounts of plant seeds (Harrison et al., 2003; Lund & Turpin, 1977). Thereby, they not only act as antagonists of insect pest species but may also contribute to plant community composition, as weed suppression (Davis et al., 2003; Honek et al., 2003; Menalled et al., 2006). With feeding on seeds they are likely to benefit from diverse plant communities because of the higher amount of seeds produced by diverse plant communities. Indeed, it has been shown that higher plant diversity in organic farming systems increases resource supply for seed feeding carabid beetles (Diehl et al., 2012; Graziani et al., 2012). Such increased food availability increase their potential to control weeds as well as insect pest species (Bàrberi et al., 2010).

Similar to carabid beetles, spiders have been shown to effectively control insect herbivore pest species (Griffin et al., 2013; Sunderland, 1999). Their wide prey spectrum allows them to occupy a variety of niches (Wise, 1993). Besides prey availability, physical habitat characteristics, such as plant architecture, determine spider species diversity and composition of spider communities (Langellotto & Denno, 2004; Uetz, 1991). In addition to providing structure for web building, the composition of plant species also affects spider assemblages indirectly via driving the abundance and composition of herbivore prey (Dennis et al., 2001). Thereby, plant species composition may

indirectly alter the control of invertebrate herbivores by spiders and in turn, top-down control of insect herbivores by spiders may alter plant community composition and e.g., enhance plant diversity (Haddad et al., 2009; Schmitz, 2003; Snyder et al., 2006).

For our study, we chose two of the most abundant predatory arthropods in central European grasslands, the ground beetle *Harpalus rufipes* (De Geer, 1774) (Coleoptera; Carabidae) and the wolf spider *Trochosa ruricola* (De Geer, 1778) (Araneae; Lycosidae). Both species are geographically widespread, locally abundant and present in many natural and agricultural ecosystems (Clough et al., 2005; Freude et al., 2004; Öberg & Ekbom, 2006). *H. rufipes* forms part of a group of large sized carabid beetles (10–17 mm) characterized by a life cycle lasting two years, autumnal breeding, nocturnal activity and good dispersal by moving on the ground but also by flying (Purtauf et al., 2004; Zhang et al., 1997). Notably, they not only feed on animal prey but also on seeds of plants (Briggs, 1965; Hartke et al., 1998, Luff, 1980). Particulary adults may be important seed predators (Gallandt et al., 2005; Harrison & Gallandt, 2012; Shearin et al., 2008). In contrast, *T. ruricola* is a generalist predator feeding exclusively on living animal prey hunted during the day. As most lycosid spiders, their prey is identified by optical and tactile cues (Edgar, 1969; Ford, 1977; Schoener, 1971) attacking virtually all mobile prey species entering their reach, however, following the attack they may reject distasteful prey (Ford, 1977; Toft & Wise, 1999b). Feeding on a variety of prey differing in food quality has been shown to beneficially affect lycosid spiders (Bernays, 1993; Toft and Wise, 1999a; Uetz et al., 1992). However, the food spectrum is assumed to be determined predominantly by prey availability (Riechert & Harp, 1987; Riechert & Lockley, 1984; Wise, 1993) and includes small soft-bodied arthropods, such as flies, springtails, bugs and spiders (Chen & Wise, 1999; Kajak, 1995; Schaefer, 1974).

Stable isotope analysis is an important and widely used tool for studying the trophic structure of animal communities and ecosystem functions (Martinez del Rio & Wolf, 2005; Post, 2002). Although used predominantly for investigating interspecific differences in trophic niches, it allows investigating both intra- and interspecific variability in trophic relationships (Layman et al., 2012; Michener & Lajtha, 2007; Oelbermann & Scheu, 2002). The nitrogen isotope ratio ($^{15}N/^{14}N$, usually expressed as $\delta^{15}N$) increases in insects by about 2.5‰ ± 1.8‰ (mean ± 1 SD) per trophic level (Ikeda et al. 2010) thereby reflecting the trophic position of species in food webs. The carbon isotope ratio ($^{12}C/^{13}C$, usually expressed as $\delta^{13}C$) changes little (about +1‰; Deniro & Epstein, 1981; France & Peters, 1997; Peterson & Fly, 1987) and it is commonly used to evaluate the source of carbon, often to distinguish between the flux of carbon fixed by C_3 plants from that fixed by C_4 plants (Peterson & Fly, 1987; Tiunov, 2007). The comparison of $\delta^{13}C$ and $\delta^{15}N$ values

within and among species allows assessing the degree of trophic overlap between species and among individuals of the same species (Gratton & Forbes, 2006; Halaj et al., 2005; Wise et al., 2006). However, this discrimination may vary depending on a consumer's nutritional status, diet quality, body size, age, dietary ontogeny and tissue and elemental composition (Ben-David & Schell, 2001; Minagawa & Wada, 1984; Vanderklift & Ponsard, 2003).

Another approach to investigate the diet of the animals is the analysis of fatty acids (FAs) as FAs can be traced from one trophic level to another, uncovering the diet of consumers and consequently food web links (Ruess et al., 2002; Ruess & Chamberlain, 2010). In consumers, most of the assimilated FAs are converted into neutral lipid FAs (NLFAs) and incorporated into the fat body which may comprise a large fraction of their body mass. Since it is energetically more efficient to incorporate FAs without modification ("dietary routing"; Blem, 1976; Pond, 1981), NLFAs of the fat body reflect the FA composition of the food sources to a significant extent (Ruess et al., 2004) and therefore allow tracing food relationships. In addition, phospholipid fatty acid (PLFA) offers information about the microbial community composition in the soil (Ramsey et al., 2006). This information can be used to study consumer - resource interrelationships. The combination of stable isotope and FA analysis allows detailed insight into trophic niches of species but also individuals and their changes in time and between ecosystems (Ferlian & Scheu, 2014; Lau et al., 2008).

We analysed variations in the diet of two of the most abundant arthropod predators in central European grasslands, i.e., the carabid beetle *H. rufipes* and the lycosid spider *T. ruricola* using natural stable isotopes and fatty acids analysis. We expected *T. ruricola* to occupy a higher trophic position than *H. rufipes* due to its strictly carnivorous diet. Further, we hypothesized the diet of *H. rufipes* to vary more with plant community composition than that of *T. ruricola* due to the omnivorous diet of the former including plant seeds.

Material and methods

Experimental design

The study site is a semi-natural temperate grassland in the Saale river floodplain near to the city of Jena (Thuringia, Germany). The site had been used as arable field for more than 40 years before the plant diversity experiment was established in 2002. The experiment comprises 80 plots of 5 x 6 m arranged in four blocks to control for changes in soil texture with increasing distance to the river. A gradient of plant species richness (1, 2, 4, 8, 16 and 60) and plant functional group

richness (1 to 4 functional groups: grasses, small herbs, tall herbs, and legumes) was established to represent a typical hay meadow in the region. Plots are mown twice a year and weeded three times per year (for details see Roscher et al., 2004).

Flooding

In May 2013, rainfall in Germany was exceptionally high; in Jena, precipitation amounted 150 mm resulting in the experimental field being flooded for 24 days (30 May to 24 June). This led to anaerobic soil conditions, as shown by redox potentials ranging from -121 to 193 mV in the soil (Wright et al., 2015). Water coverage was measured for each plot daily from 31 May to 24 June and ascribed to five levels: 0, 25, 50, 75, and 100%. Flooding severity was evaluated using a continuous flooding index (sum of the percentage water coverage per day over the flooding period; Wright et al., 2015). Flooding index was used as explanatory covariable to account for possible bias caused by flood intensity.

Sampling

H. rufipes and *T. ruricola* were collected using pitfall traps in August 2013. Two 4.5 cm diameter pitfall traps containing 3% formalin were placed in each plot. Animals were collected every two weeks, stored in 70% ethanol (for details see Ebeling et al., 2014) and identified to species (Engelhardt, 1964; Schaefer, 2010). Because of a high variability body size of *T. ruricola,* individuals were divided into small (from 0 to 0.4 cm) and large (from 0.5 to 1.0 cm) ones. *H. rufipes* individuals were highly homogenous in their body size.

Stable isotope analysis

Prior to analysis of natural variations in $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios, *T. ruricola* and *H. rufipes* specimens were dried at 105°C for 48 h and then ground using a mortar and pestle. Ground animal tissue was transferred into tin capsules (one individual per sample), weighed and $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios measured using an elemental analyser (NA 1500, Carlo Erba, Milano, Italy) coupled with a mass spectrometer (MAT 251, Finnigan, Bremen, Germany; Langel & Dyckmans, 2014; Reineking et al., 1993). PD belemnite and atmospheric nitrogen served as primary standard for ^{13}C and ^{15}N, respectively. Acetanilide (C_8H_9NO, Merck, Darmstadt, Germany) was used for internal calibration. Isotope natural abundance was expressed by the delta notation with $\delta^{13}C$ or $\delta^{15}N$ [‰] = $(R_{sample} - R_{standard})/(R_{standard} \times 1000)$, where R is the ratio of the heavier isotope to the lighter isotope.

Inorganic carbon typically is isotopically heavy as compared to organic carbon and positively skews $\delta^{13}C$ values of animals if they contain carbonate-rich skeletons (Fry, 1988; Haines & Montague, 1979). In order to remove inorganic carbon, prior to stable isotope analysis, *H. rufipes* samples were also analysed after adding 0.1 N HCl (Hobson et al., 2002).

The data were checked for normality and homoscedasticity using Shapiro - Wilk and Fligner - Killeen tests. The natural $\delta^{13}C$ and $\delta^{15}N$ values were used as dependent variables. Linear models (analysis of variance (ANOVA), type I sum of squares) were used to analyse the effect of block (categorical variable, four blocks), body size of the animal (small and large; only in *T. ruricola*), flooding index (continuous variable, from 1 to 23), plant diversity (continuous variable, from 1 to 60; log-transformed, Hooper et al., 2005), and presence/absence of legumes, grasses, small herbs and tall herbs (categorical variable) including interactions. The Akaike Information Criterion (AIC) was used to select the best model by dropping non-significant variables from the full model in a step wise manner (Faraway, 2014; Zuur el al., 2007). The linear model had a hierarchical order: Block and flooding index were fitted first followed by body size and plant species richness; thereafter, presence/absence of grasses, legumes, tall herbs and short herbs were fitted. Afterwards, F-values given in text and tables refer to models in which the respective factor was fitted first following the order mentioned before (Schmid et al., 2002). Block and flooding index were fitted first followed by body size and plant species richness; thereafter, presence/absence of grasses, legumes, tall herbs, and short herbs were fitted. Statistical analyses were performed using R 3.2.1 (R Development Core Team, 2014; http://www.R-project.org).

Neutral fatty acid analysis

T. ruricola and *H. rufipes* specimens were cleaned to remove particles attached to the body surface before extraction of total lipids from each individual, which were then extracted and fractionated into neutral lipids as described in Haubert et al. (2004). The neutral lipid fraction was dried in a rotation vacuum concentrator (50°C), and the lipids saponified, methylated and washed following the protocol for the Sherlock Microbial Identification System (MIDI Inc., Newark, USA). Fatty acid methyl esters (FAMEs) were transferred into small vials, capped and analysed by gas chromatography (CLARUS 500, Perkin Elmer, Waltham, USA). The analysis system used was equipped with a flame ionization detector (PE-5 capillary column, 30 m × 0.32 mm i.d., 0.25 mm film thickness, Perkin Elmer, Waltham, USA); helium was used as carrier gas. FAMEs were identified by comparing retention times of samples and standard mixtures comprising unbranched and branched (bacterial) FAMEs. The following fatty acids served as biomarkers for

bacteria: the methyl- branched FAs i15:0, a15:0, i16:0 and i17:0 (Gram-positive) and the cyclic FAs, cy17:0 and cy19:0 (Gram-negative). The unsaturated FAs 18:1ω9 and 18:2ω6,9 served as relative plant and fungal markers, respectively (Ruess & Chamberlain, 2010). Moreover, 16:2ω6,9 and 16:3ω3,6,9 are used as biomarkers of green algae (Chlorophyceae) (Buse et al., 2013). Monoenic C20 in their FA profile is an indication of a eukaryote diet (Ruess et al., 2004). All other markers, such as 10:00 and 12:00, cannot be assigned to specific taxa and are called unspecific markers.

Percentage values of NLFAs and PLFAs were logit-transformed and non-metric multidimensional scaling (NMDS) was used to compress the data. MANOVA was performed using the scores of the NMDS axes. Thereafter, if significant, differences within individual FAs were inspected by single factor ANOVA. Principal components analyses (PCAs) of logit-transformed mole percentage values were performed using Canoco 5 (Ter Braak & Smilauer, 2012). As supplementary variables were used flooding index, plant functional group, plant species richness and the presence of legumes, grasses, tall herbs and small herbs. Pearson's correlations were used to evaluate the relationship between logit-transformed marker FA concentrations [sum of bacterial markers, fungal marker (18:2ω6,9) and plant marker (18:1ω9)] in soil as compared to *H. rufipes* and *T. ruricola*.

Phospholipid fatty acid analysis

Phospholipids (PLFAs) were extracted from soil taken in September 2013 (93 days after the natural flood). Three soil cores of 2 cm diameter were taken from 0-10 cm soil depth and were pooled to form one composite sample. Afterwards soil was sieved with 2 mm mesh size to remove stones and animals. PLFA extraction followed the protocol by Frostegård et. al. (1991) with modifications as mentioned by Wagner et. al. (2015) using 5 g of fresh soil. The analysis system used (GC 17A, SHIMADZU, Kyoto, Japan) was equipped with column DB 225MS (60 m × 0.25 mm i.d., 0.25 μm) and an autosampler (AOC 5000, SHIMADZU). Hydrogen was used as carrier gas. The software GC Lab Solution (SHIMADZU) were used to assign peaks to fatty acids by comparing FAMEs to retention times of samples and standard mixtures comprising unbranched and branched (bacterial) FAMEs. The same biomarkers as mentioned for NLFA analysis were used.

Results

Harpalus rufipes

The δ^{15}N signature of *H. rufipes* was on average 5.88‰, but the values were highly variable and ranged from 3.52 to 8.63‰ spanning 5.11 δ units (Fig. 1). δ^{15}N signatures decreased significantly with increasing plant species richness (Fig. 2), whereas they were slightly increased in the presence of legumes (Table 1). The δ^{15}N signatures did not vary significantly with flooding index. Average δ^{13}C signature of *H. rufipes* was -23.92‰, but again values were highly variable, ranging from -18.28 to -29.21‰, i.e. spanning over 10.93 δ units (Fig. 1). δ^{13}C signatures significantly increased in the presence of grasses and small herbs, but were not significantly affected by other plant community properties and flooding index (Table 1).

The FA composition of *H. rufipes* was significantly affected by the presence of legumes and marginally by the presence of grasses (Table 2). Concentrations of the plant marker 18:1ω9 increased significantly with the presence of grasses and marginally with the presence of legumes (Table 3). Similarly, the unspecific FA marker 18:00 and the eukaryote FA markers 20:4ω6 and 20:1ω9 increased significantly in the presence of legumes. The unspecific marker FAs 16:00 (significantly) and 18:1ω7 (marginally) increased in the presence of grasses, whereas the unspecific FA 23:00 decreased significantly (Table 3).

Bacterial marker FAs in *H. rufipes* were negatively correlated with the bacterial marker PLFAs in soil (Table 4). In contrast, the plant FA marker in *H. rufipes* (18:1ω9) was positively correlated with the plant PLFA marker in soil. There was no correlation with the fungal FA marker in *H. rufipes* and the plant PLFA marker in soil (Table 4).The first four PCA axes explained 51.76 % of the total variation in the FA composition of *H. rufipes*, with the first axis accounting for 20.92 % and the second axis for 11.11% (Fig. 3). The first axis was separated by bacterial markers vs fungal and plant markers. Further the plant marker seems to correlate negatively with flooding index, plant functional group and species richness. The first axis was related to the presence of small herbs, whereas the second axis was associated with the presence of legumes. Concentrations of the bacterial marker cy17:0 and a15:0 and unspecific markers 15:00 and 17:00 increased in the presence of small herbs. Concentrations of unspecific FA markers such as 16:1ω5 and 22:00 were higher in presence of legumes.
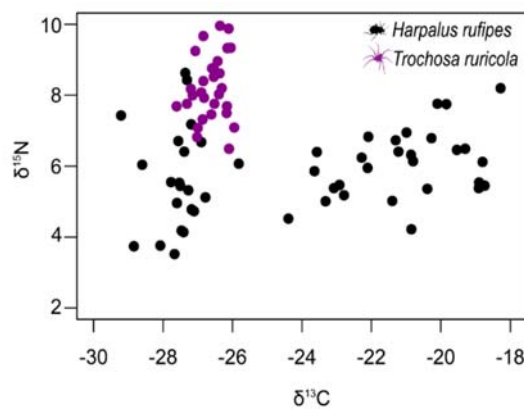
Fig. 1. Variations in δ^{15}N and δ^{13}C signatures of *Harpalus rufipes* (black points) and *Trochosa ruricola* (purple points) across the plots of the Jena Experiment.
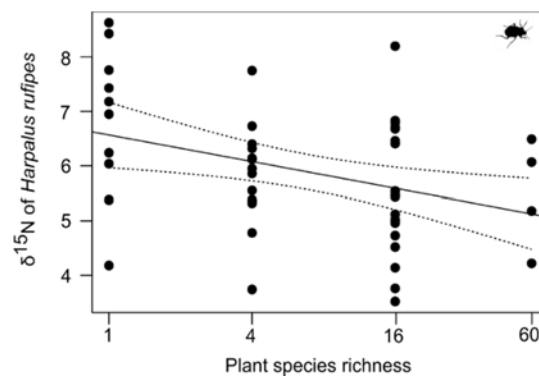

Fig. 2. Variations in δ^{15}N signatures on *Harpalus rufipes* ($P < 0.01$; $R2 = 0.11$) as affected by plant species richness (log-transformed).
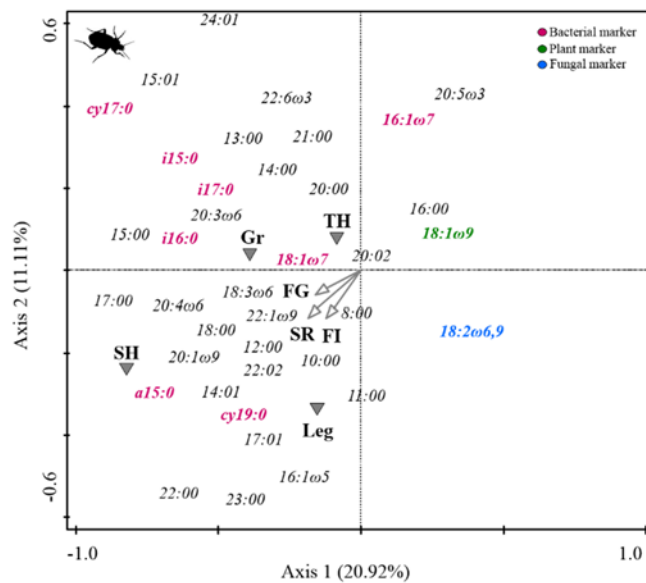

Fig. 3. Principal components analysis of the relative abundance (mol%, logit- transformed) of individual NLFAs of *Harpalus rufipes* using flooding index (FI), plant species richness (SR), plant functional group (FG), presence of grasses (Gr), legumes (Leg), small herbs (SH) and tall herbs (TH) as supplementary variables. Bacterial markers in pink, plant marker in green and fungal marker in blue.

Trochosa ruricola

The δ^{15}N signature of *T. ruricola* was on average of 8.22‰, but the values were variable and ranged from 6.49 to 9.96‰ (Fig. 1). However, the range (3.47 δ units) was considerably lower than that in *H. rufipes*. The δ^{15}N signatures of *T. ruricola* significantly increased with body size and flooding index (Fig. 4; Table 1). Average δ^{13}C signature of *T. ruricola* was -26.60‰. Contrasting *H. rufipes*, δ^{13}C signatures of *T. ruricola* only varied over 1.66 δ units, i.e., from -25.95 to -27.61‰ (Fig. 1). None of the studied factors significantly affected the δ^{13}C signatures of *T. ruricola* (Table 1).

The FA composition of *T. ruricola* varied significantly with body size (Table 2). The eukaryote marker FAs 20:4ω6 and 20:5ω3 significantly increased with body size (Table 3). Further, the fungal marker FA 18:2ω6,9 increased marginally with body size. Unspecific marker FAs 10:00 and 12:00 decreased significantly, while bacterial FA 18:1ω7 increased significantly with body size (Table 3). The FAs 10:00 and 16:00 decreased in the presence of small herbs. Further, the unspecific FAs 16:00 (significantly) and the bacterial marker FA 16:1ω7 (marginally) decreased in presence of tall herbs. FAs of *T. ruricola* were not correlated significantly with any PLFA markers in the soil (Table 4).

The first four PCA axes explained 67.40 % of the total variation in the dataset, with axis 1 accounted for 30.55 % and axis 2 for 15.44 % (Fig. 5). The first axis of the PCA reflected variations in FAs of *T. ruricola* with body size (small or large), with the concentration of eukaryote markers 20:4ω6 and 20:5ω3 increasing in larger individuals. In contrast, small individuals contained higher concentrations of unspecific FAs such as 12:00 and 10:00. The second axis of the PCA was related to the number of plant functional groups and the presence of small herbs, but the effect is rather small. Concentration of the FAs 18:2ω6 and 18:3ω6 increased at sites with higher number of plant functional groups and in the presence of small herbs.

Table 1. F-value table of linear models on the effect of block, body size (Size), flooding index (FI), plant species richness (SR), presence of grasses (Gr), legumes (Leg), small herbs (SH) and tall herbs (TH) on the $\delta 15N$ and $\delta 13C$ signatures of *Harpalus rufipes* and *Trochosa ruricola*. Significant effects (P < 0.05) are highlighted in bold and marginally significant results (P < 0.10) are given in italics. ↑ Increase with increasing plant species richness level, body size, flooding index, or in presence of the respective plant functional group, ↓ decrease. Factors without F and P-values were excluded from the final models.

	Harpalus rufipes				*Trochosa ruricola*			
	$\delta^{15}N$		$\delta^{13}C$		$\delta^{15}N$		$\delta^{13}C$	
Factors	df	F	df	F	df	F	df	F
Block	-		3,49	**4.37**	-		*3,29*	*2.32*
Size	-		-		1,29	↑ **31.66**	-	
FI	-		-		1,29	↑ **3.70**	-	
SR	1,50	↓ **7.73**	-		-		-	
Gr	-		1,49	↑ **5.18**	-		-	
Leg	1,50	↑ *3.44*	-		-		-	
SH	-		1,49	↑ **4.33**	-		-	
TH	-		-		-		-	

Fig. 4. Variations in $\delta^{15}N$ signatures of *Trochosa ruricola* as affected by its body size (small, large; P < 0.01) and flooding index (P = 0.06; R2 = 0.12).
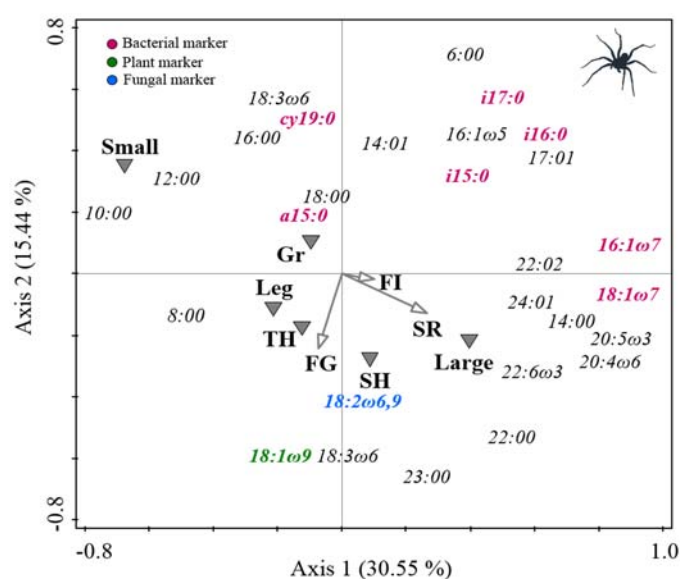
Fig. 5. Principal components analysis of the relative abundance (mol%, logit- transformed) of individual NLFAs of *T. ruricola* using body size of *T. ruricola* (Small/Large), flooding index (FI), plant species richness (SR), plant functional group (FG), presence of grasses (Gr), legumes (Leg), small herbs (SH) and tall herbs (TH) as supplementary variables. Bacterial markers in pink, plant marker in green and fungal marker in blue.

Phospholipid fatty acids in soil

The MANOVA results from NMDS indicated block (significantly), flooding index (marginally) and tall herbs (marginally) to affect the composition of PLFAs in soil (Table 2). The bacterial marker i16:0 and the plant marker 18:1ω9 increased significantly in heavily flooded plots (Table 3). By contrast, concentrations of the fungal marker 18:2ω6,9 significantly decreased at higher flooding index. The unspecific marker FAs 17:00 increased significantly at higher flooding index, whereas FAs 18:00 and 21:00 significantly decreased. The bacterial marker cy19:0 and the unspecific FA marker 16:00 increased significantly in the presence of tall herbs (Table 3).

The first four PCA axes explained 83.04 % of the total variation in PLFAs. The first axis accounted for 39.23 % and the second axis for 20.23% (Fig. 6). The distribution of the FAs varied with plant functional groups. The first axis represented the presence of grasses and legumes, whereas the second axis represented the presence of tall herbs and small herbs. In presence of tall herbs concentrations of the eukaryote marker FA 21:00 and the bacterial marker cy17:0 increased, whereas in the presence of small herbs the bacterial FA marker 19:00 increased (Fig. 6).
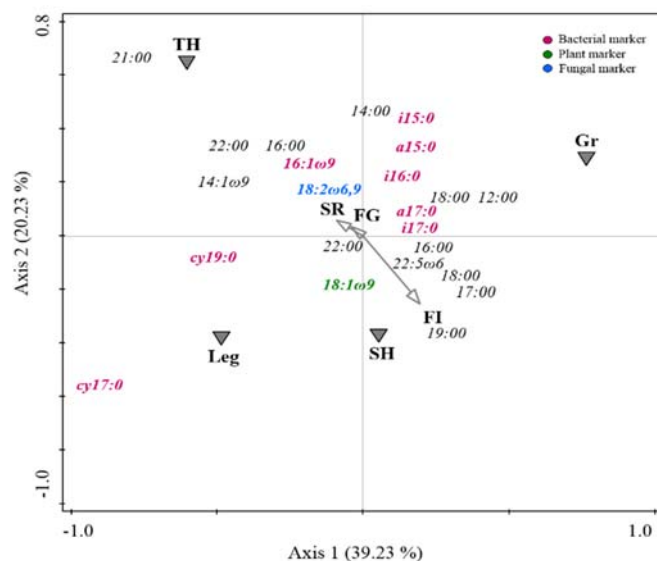
Fig. 6. Principal components analysis of the relative abundance (mol%, logit- transformed) of individual PLFAs of the soil using flooding index (FI), plant species richness (SR), plant functional group (FG), presence of grasses (Gr), legumes (Leg), small herbs (SH) and tall herbs (TH) as supplementary variables. Bacterial markers in pink, plant marker in green and fungal marker in blue.

Table 2. MANOVAs of the axes of the NMDS of neutral fatty acid composition of *T. ruricola*, *H. rufipes* and phospholipid composition (PLFAs) of soil indicating the effect of block, body size (only in *T. ruricola*), flooding index (FI), presence of grasses (Gr), legumes (Leg), small herbs (SH) and tall herbs (TH). Significant effects (P < 0.05) are highlighted in bold and marginally significant results (P < 0.10) are given in italics. ↑ increase ,↓ decrease. Factors without F and P-values were excluded from the final models.

| | *H. rufipes* | | *T. ruricola* | | PLFA | |
	d.f	F	d.f	F	d.f	F
Block	3,45	**2.17**	-		3,45	**2.82**
Size	-		1, 46	**5.31**	-	
FI	-		-		1,45	*2.51*
Sh	-		1, 46	*2.32*	-	
Th	-		1, 46	*2.27*	1,45	*2.61*
Leg	1,45	**3.28**	-		-	
Gr	1,45	*2.47*	-		-	

Table 3. ANOVA table of F-values (with degrees of freedom) on the effects of the significant factors of legumes (Leg), grasses (Gr), size (Size), small herbs (Sh), tall herbs (Th) and flooding index (FI)(Table 2) on neutral fatty acids of *Trochosa ruricola*, *Harpalus rufipes* and on phospholipid fatty acids (PLFAs) of soil on amounts of FAs and PUFAs (20:4 ω 6, 20:5 ω 3, 20:2 ω 6). Factors without F and P-values were excluded from the final models. Bacterial markers in pink, plant marker in green and fungal marker in blue.

	H. rufipes			*T. ruricola*		*PLFA*	
FA	Leg ($F_{1,45}$)	Gr ($F_{1,45}$)	Size ($F_{1,46}$)	Sh ($F_{1,46}$)	Th ($F_{1,46}$)	FI ($F_{1,45}$)	Th ($F_{1,45}$)
8:00	-	-	-	↓3.93	-	-	-
10:00	↑3.33	-	↓12.91	↓5.34	-	-	-
12:00	-	-	↓17.22	-	-	-	-
i16:0	-	-	-	-	-	↑5.31	-
16:00	-	↑4.10	-	↓3.28	↓4.59	-	↑5.31
16:1ω7	-	-	-	-	↓3.45	-	-
i17:0	-	-	-	-	-	-	↓3.54
17:01	-	-	-	-	-	↑3.05	↓3.16
cy17:0	-	-	-	-	-	↑4.05	-
17:00	-	-	-	-	-	↑6.49	-
18:3ω6	↑3.52	-	-	-	-	-	-
18:2ω6,9	-	-	↑3.97	-	-	↓4.14	↑3.69
18:1ω9	↑3.20	↑6.28	-	-	-	↑4.63	-
18:1ω7	-	↑3.35	↑5.45	-	-	-	-
18:00	↑6.04	-	-	↓4.68	-	↓6.44	-
cy19:0	-	-	-	-	-	-	↑5.22
20:4ω6	↑4.41	-	↑11.11	↑3.10	-	-	-
20:5ω3	-	-	↑24.70	-	-	-	-
20:1ω9	↑5.39	-	-	-	-	-	-
21:00	-	-	-	-	-	↓6.10	↑4.05
23:00	-	↓4.39	-	-	-	-	-

Table 4. Pearson correlation coefficients of regressions between logit-transformed marker phospholipid marker (PLFA) concentrations and sum of bacterial markers (see Materials and methods), fungal marker (18:2ω6,9), and plant marker (18:1ω9) of soil in comparison to neutral fatty acids in *H. rufipes* and *T. ruricola*. Significant effects are given in bold.

		PLFA markers		
		Bacterial	Plant	Fungal
H. rufipes markers	Bacterial	**-0.45**	**0.48**	0.01
	Plant	0.07	-0.03	-0.15
	Fungal	-0.06	0.02	0.16
T. ruricola markers	Bacterial	-0.03	0.04	0.03
	Plant	0.10	-0.11	0.03
	Fungal	0.07	-0.05	-0.10

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Discussion

As we expected, stable isotope analysis suggests that *T. ruricola* occupies a higher trophic position than *H. rufipes*. As indicated by fatty acid analysis, the diet of *H. rufipes* includes plant seeds. The more variable diet of *H. rufipes* also is reflected by the wider range of stable isotope values as compared to *T. ruricola*, which is only feeding on animal prey, pointing to an omnivorous diet with substantial contribution of plant seeds.

As we hypothesized, the diet of *H. rufipes* varied significantly with plant community composition. Its diet markedly depended on the available plant resources, as indicated by the close correlation between the FA plant marker in the soil and in body tissues. *H. rufipes* consumed more plant resources in more complex and species-rich plant communities. Seed predation by insects has been shown to increase with increasing plant species richness (Preukschas et al., 2014; Vockenhuber et al., 2013). The phenology of plants differs among species, and therefore more diverse communities provide seeds over a longer period of time presumably benefiting *H. rufipes*. In fact, beetles of the genera *Harpalus* were shown to become more abundant in the presence of higher numbers of weed plants in agricultural fields (Kokta, 1988; de Snoo et al., 1995). Also, *H. rufipes* has been shown to benefit from a diet comprising a diversity of seeds rather than only seeds of one plant species (Bilde & Toft, 1994; Brygadyrenko & Reshetniak, 2014; Wallin et al., 1992). Indeed, seeds are high food quality for granivorous carabid beetles (Fawki & Toft, 2005).

In the present study, the presence of grasses and small herbs increased the $\delta^{13}C$ signatures of *H. rufipes*. In the Jena Experiment, grasses produce their seeds earlier during the vegetation period than most tall herbs and legumes and generate larger amounts of seeds than herbaceous plants (C. Roscher, pers. comm.). These may explain the increased contribution of plants (seeds) to the diet of *H. rufipes*. Moreover, the availability of grass seeds substantially exceeded that of herbaceous plants such as legumes because grasses produce their seeds earlier during the vegetation period than most tall herbs and legumes (C. Roscher, pers. comm.). Besides, fatty acid analyses indicated that in the presence of legumes the diet of *H. rufipes* shifted towards more animal prey, presumably including aphids and collembolans (Kielty et al., 1999). Legumes are considered key plant species (Milcu et al., 2008), which may suffer strongly from herbivore attack (Loranger et al., 2014). Further, legumes favour detritivore soil invertebrates by providing nitrogen-rich litter material (Spehn et al., 2002). Indeed, in the Jena Experiment the density and diversity of Collembola increased in the presence of legumes (Sabais et al., 2011) and Collembola

are important prey of carabid beetles, in particular of juveniles (Eitzinger & Traugott, 2011; Mundy et al., 2000).

The δ^{13}C values of *H. rufipes* spanned a markedly wide range including values considerably higher than those of C$_3$ plants suggesting that its body carbon originated from food sources other than the C$_3$ plants dominating at the study site. Primary producers characterized by high δ^{13}C values include algae (Akamatsu et al., 2004; Ruess et al., 2004) and potentially *H. rufipes* may have fed on algivore prey species such as soil surface dwelling Collembola (Potapov et al., 2018), but no algae FA markers were detected in *H. rufipes*. Further, high δ^{13}C values may have been due to *H. rufipes* feeding on seeds of C$_4$ plants or on herbivores feeding on C$_4$ plants. Although not included in our sampling regime, there are two plots of 10 x 20 m with a C$_4$ plant species (*Amaranthus retroflexus* L.) in the Jena Experiment. For identifying if seeds or herbivores of these plants may have contributed to the diet of *H. rufipes* we investigated if the enrichment in δ^{13}C values in *H. rufipes* was more pronounced close to the two *A. retroflexus* plots. In fact, however, distance to the *A. retroflexus* plots did not significantly correlate with δ^{13}C values in *H. rufipes* arguing against this explanation. Nevertheless, this does not rule out this explanation as carabid beetles such as *H. rufipes* are able to fly long distances (Zhang et al., 1997) and also move fast on the soil surface in a random way (Galis & Jong, 1988). Although *A. retroflexus* produces small seeds, feeding efficiency (i.e., seeds eaten per distance travelled) in *H. rufipes* is particularly high for seeds of this species (Harrison & Gallandt, 2012). Moreover, seed production of *A. retroflexus* is excessive with 5000 to 300,000 seeds per plant (Costea et al., 2004), suggesting that the observed high δ^{13}C values in *H. rufipes* may have been due to feeding on plants of this species.

In contrast to *H. rufipes*, the diet of *T. ruricola* did not vary with plant species richness, being more independent of the diversity and structure of plant communities than *H. rufipes*, suggesting that it may also be rather independent of variations in environmental factors and plant associated prey communities (Ebeling et al., 2017). Rather, it changed markedly with body size, indicating dietary changes during developmental stages, from first towards secondary predators (e.g. decomposers and predators with higher δ^{15}N values). Larger juveniles and adults of *T. ruricola* occupy higher trophic positions in the food web than small juveniles, suggesting that with increasing body size intraguild predation or cannibalism play a larger role than exploitive competition (König et al., 2011; Schneider, 2012). Spiders are known to be voracious intraguild predators (Denno et al., 2004; Lensing & Wise, 2004). Indeed, the larger the top predator the more it is feeding on larger other predator species (Riede et al., 2011; Schneider et al., 2012). As a consequence, larger predators may switch from exploitative competition with similar sized

predators over intraguild predation to ocuppaying higher trophic level with the largest predator at the top. High $\delta^{15}N$ values in *T. ruricola* adults therefore may reflect its position as second order predator. Notably, in severely flooded plots, the $\delta^{15}N$ values of *T. ruricola* were higher than in plots little affected by the flood. Earlier studies showed that flooding decreased bacterial biomass, whereas it increased fungal biomass, reflecting the elevated availability of dead plant biomass in severely flooded plots (Wagner et al., 2015; Wright et al., 2015), which probably increased the abundance of detritivore prey to *T. ruricola*. Overall, therefore, the diet of *T. ruricola* may have consisted mainly of secondary decomposers such as fungal feeding Collembola in severely flooded plots as also reflected by fatty acid biomarkers.

Conclusions

Carabid beetles and spiders are among the most abundant and important predators in grasslands. Stable isotope and fatty acid analysis of *H. rufipes* and *T. ruricola* yielded novel insights into factors driving trophic interrelationships of these species and their role in controlling herbivores. Notably, the study allowed investigating the role of variations in plant species richness, plant functional group richness and presence/absence of functional groups of plants on intraspecific dietary variation of these predator species. The results indicate that the diet of *H. rufipes* varied with the availability of plant resources and increased with increasing plant diversity as well as in the presence of grasses and small herbs. In contrast, presence of legumes increased the consumption of animal prey, presumably aphids and collembolans. In contrast to *H. rufipes*, the diet of *T. ruricola* comprised only animal prey and was independent of plant species richness. Interestingly, however, it varied markedly with body size with larger individuals occupying higher trophic positions as second order predators. Moreover, the diet of *T. ruricola* changed due to flooding, presumably by increased availability of secondary decomposers as well as intraguild prey in severely flooded plots. Our results reinforce the view that there are feedback loops between plant and invertebrate predator communities with more effective control of pest species in more diverse plant communities. Notably, generalist predators of different taxonomic and functional groups, such as carabid beetles with a wide diet including also seeds and wolf spiders with animal prey only, are likely to complement each other in controlling insect herbivore species and thereby herbivore–plant interactions.

Acknowledgements

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Chapter 5

General Discussion

Photos from the Jena Experiment

Anne Ebeling, Odette González Macé and .www.idiv.com, respectively.

General discussion

This thesis is a unique study of a natural and infrequent flood event inside a biodiversity experiment. I studied the effects of the flood into the aboveground-belowground food web including the variation of different plant diversity and plant functional group. I hypothesized that the flood is as a large and infrequent disturbance (LID) and, in consequence, it will decrease the abundance and diversity of communities and will create heterogeneous pattern of surviving organisms (Hypothesis 1). Moreover, I expect plant diversity will foster the recovery of the food web (Hypothesis 2). I hypothesized aboveground-belowground food web will be influenced drastically by the flood. Basal soil food web components will be more affected by flooding than higher trophic levels due to lower dispersal ability. Also, I expect the pattern of Collembola and Oribatida species richness will follow the Intermediate Disturbance Hypothesis, when the highest species diversity occur at intermediate levels of flooding (Hypothesis 3).

Hypothesis 1: large and infrequent disturbance

Following my expectation (Hypothesis 1), the flooding in 2013 in the Jena Experiment was a large and infrequent disturbance (LID) event, which influenced the examined soil food web within a short time (i.e. three weeks) but not for long. However, flooding changed soil abiotic conditions with a significant magnitude. First, in the most severe flooded plots an anaerobic condition was developed (Wright et al., 2015). Second, N-rich sediments were brought by the flood and left in the field, especially accumulated at the plots with higher plant diversity due to the complex plant structure. In addition, mortality of soil animals and plants in the plots with high plant diversity was high (N. Eisenhauer, personal observation) and therefore likely increased nutrient availability resulted from decomposition of the dead tissues. In consequence, degradation processes of chitin and lignin were fostered and C and P limitation was increased. All this abiotic environmental changes may have downstream effects on diversity and abundance of soil communities (Abgrall et al., 2017).

The hypothesis assumes that the abundance and diversity of communities across the field will decrease and spatial heterogeneity will increase due to the flood (Turner et al., 1998). Although almost all microbial functions were recovered, the Collembola and Acari communities were not recuperated three weeks after the flood. The communities decreased in abundance and diversity as predicted. Furthermore, the 2013 flood homogenized environmental conditions of the Jena experimental plots and eradicated the effects of plant community composition on soil biota established before. However, the flood in the Jena Experiment was a special case and may not be

applied to other cases where flood may still contribute to spatial heterogeneity, because the plots of the Jena Experiment were already characterized by their high spatial heterogeneity before the flood (due to the established plant diversity gradient; Roscher et al., 2004).

Hypothesis 2: plant diversity

The response of soil communities to the flood varied reflecting legacy effects of plant diversity (de Vries et al., 2012). Species identity and community composition of plants can strongly influence composition and function of the soil food web by modifying resource inputs into the soil, thereby affecting resistance and resilience of soil biota (Bezemer et al., 2010; Eisenhauer et al., 2010; Porazinska et al., 2003). Plants growing in species-rich communities were less negatively affected by the flood than monocultures (Wright et al., 2017). In high plant diversity plots nutrient limitation was less pronounced than in monocultures, and this was associated with higher density of roots and soil animals (Ravenek et al., 2014; Scherber et al., 2010). While monocultures profited more from the extra nutrients brought by the flood, higher plant diversity communities contained more dead organic material accumulated after the flood. Soil organisms took advantage of this dead organic material. Also, plant functional groups played a role in the effects of the flood. The presence of grasses benefited the communities from nitrogen-rich sediments but not the communities with legumes. Presumably, this reflects strong N limitation in non-legume plots before the flood. However, legumes were more affected by the flood (Wright et al., 2017) and their positive effects on soil organisms disappeared after the flood. Importantly, flooding duration varied between plots of the experiment. It formed a gradient (from 1 to 24 days), which created different successional stages and spatial variability. Plots with less flooding severity and higher survival rate could act as *biological legacies* or *residuals* of soil biota. Residuals influence successional dynamics after disturbances (Del Moral & Bliss, 1993; Franklin et al., 1985; Michener & Haeuber, 1998). They enable resiliency in patches that have been subjected to a severe disturbance, help the communities to return to normal states and potentially also encourage colonization of species (Starzomski & Srivastava, 2007).

Hypothesis 3: aboveground-belowground food web

Contrary to my expectation, the aboveground-belowground food web was not influenced drastically by the flooding in the short-term and higher trophic levels were more strongly affected than basal levels. Soil microbial communities may sensitively respond to disturbances (Allison & Martiny, 2008). However, microbial biomass and basal respiration almost recovered within three weeks after flooding. Microbial communities may have high resistance and resilience against

flooding. First, dormancy is an example of how microorganisms may survive flooding. Dormancy allows organisms to enter a state of reduced metabolic activity (Jones & Lennon, 2010; Lennon & Jones, 2011). After the disturbance, surviving individuals may grow quickly to pre-disturbance population sizes if conditions are favourable. Moreover, some microbial groups show a high degree of metabolic flexibility and physiological tolerance helping them to resist environmental changes (Meyer et al., 2004). Second, microorganisms generally feature rapid growth, high population densities and high mutation rates and are capable of recombination via lateral gene transfer, which facilitates recovery after disturbance events (e.g., Lenski & Bennett, 1993). Few surviving individuals could grow quickly to recover pre-disturbance population sizes after a sudden pulse, especially if the disturbance increases resource availability (Shade et al., 2012). In the present study, soil nitrate concentration increased and, as a consequence, limitation of P and C immediately increased after the flood.

Compared to microorganisms, higher trophic levels such as Collembola and Acari communities may be more sensitive to disturbances and changes in these communities may cascade to predators of the soil food web. Even though Collembola can tolerate short periods of inundation (Zinkler & Rüssbeck, 1986), longer periods can reduce Collembola densities drastically. In the Jena Experiment, Collembola density was affected by the flood independently of the level of disturbance and plant diversity. Collembola individuals were absent in 51 from 80 plots three weeks after the flood. In contrast to my hypothesis, Collembola species richness can not be predicted by the Intermediate Disturbance Hypothesis (IDH; Connell, 1978; Pickett & White, 1985; Wootton, 1998). Collembola richness had an arbitrary pattern and it is independently of the level of disturbance (Fig. 1A). However, populations were very resilient (Russell et al., 2002) and the density and richness of Collembola were recovered three months after the flood. Different mechanisms may be responsible for fast recovery of Collembola communities of intensely flooded sites: passive dispersal (Coulson et al., 2002), immigration from peripheral areas (Rusek, 1992) or hatching from eggs which survived flooding (Gauer, 1997). Community composition varied less than expected, suggesting the community was already adapted to disturbances, but there is still some succession processes going on.

Inside the Acari communities, Oribatida are generally sensitive to disturbances due to low reproduction rate and low mobility, causing a slow recolonization of disturbed habitats (Maraun & Scheu, 2000). However, the sensitivity varies considerably among oribatid mite groups forming a gradient from high (Enarthronota) to low sensitivity (Punctoribates and Tectocepheus). Although most oribatid mites are terrestrial, many of them can survive for long time periods of

flooding (Pfingstl, 2013; Schatz & Behan-Pelletier, 2008). In the Jena Experiment, Oribatida species survived the flood better than Collembola. Oribatida individuals were absent in 27 from 80 plots three weeks after the flood. As suggested, Oribatida species richness had a tendency to follow the prediction of IDH (Fig. 1B). The highest species richness was found at intermediate intensity disturbance (between 5 to 20 days under water). Probably because at intermediate disturbance intensity more niches were created after the flood. At low disturbance intensity (5 days under water), competitive exclusion could occur and less niches were available, limiting the number of species present. High intensity disturbance, by contrast, is likely to detrimentally affect even flood resistant species, again resulting in low species richness (from 20 days under water). However, some parthenogenetic species of Oribatida can recolonize rapidly disturbed habitats due to high fecundity and fast development (Norton et al., 1993; Scheu & Schulz, 1996). Therefore, three months after the flood the density and richness were recovered to the previous states and the community species composition did not change sustancially, suggesting a high resilience of soil mesofauna after the flood.
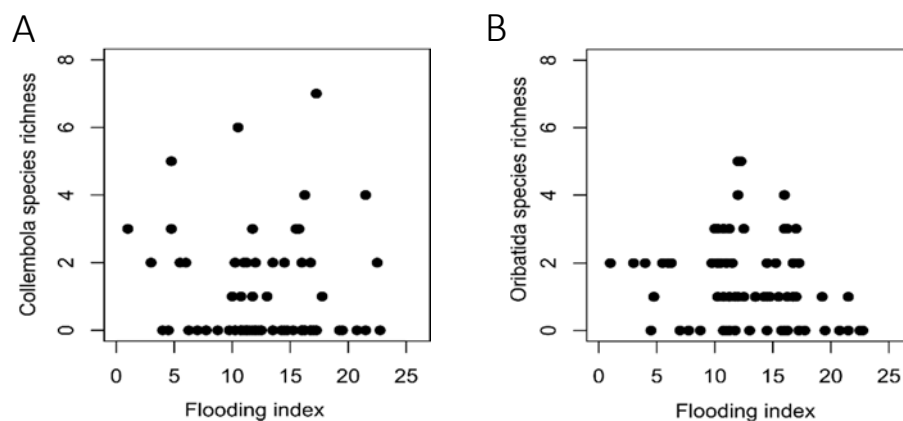
Fig. 1. Species richness of (A) Collembola (B) Oribatida as affected by flooding index.

Effects of the flood propagated up to the predator level. Cagnolo et al. (2009) found that fragmentation as disturbance caused specialists to experience a decrease in abundance while generalists were less affected due to new foraging opportunities in the form of open niches and new habitat patches. Two generalist predators of grasslands were studied. Three months after the flood, the effects of the flooding were still present in the spider species *T. ruricola* but not in the ground beetle *H. rufipes*. $\delta^{13}C$ and $\delta^{15}N$ values of *T. ruricola* decreased in the samples taken in the same month of the following year after the flood (2014; O. González, unpubl.data, Fig.2A) as compared to three months after the flood. This suggests that the prey consumed by spiders three months after the flood had higher $\delta^{13}C$ and $\delta^{15}N$ values as compared to the prey consumed

one year after the flood. Presumably, the aboveground litter material channel was more affected by the flood than the channel based on root resources, due to the input of flood sediments. Therefore, the diet of *T. ruricola* may have mainly consisted of secondary decomposers such as fungal feeding Collembola in severely flooded plots as it is also reflected by fatty acid biomarkers. Flattening of the vegetation by flooding may as well have allowed *T. ruricola* to access more prey on shoots of plants including predatory species, contributing to higher δ^{15}N values. In contrast, ground beetles *H. rufipes* did not respond to the flooding. The δ^{13}C and δ^{15}N values of *H. rufipes* did not variate between years (Fig. 2B). Rather, their diet markedly depended on the available plant resources and they consumed more plant resources in more complex and species-rich plant communities (Brygadyrenko & Reshetniak, 2014).
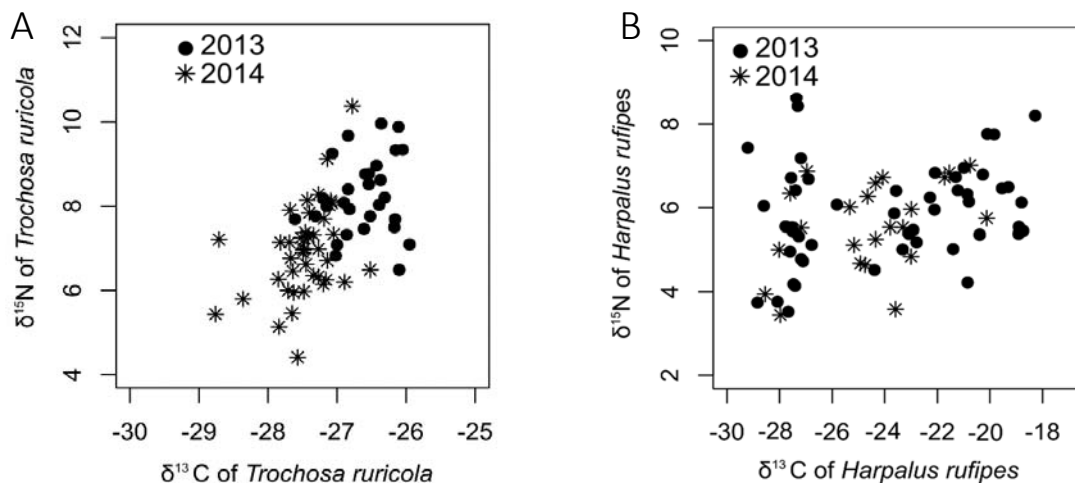
Fig. 2. Variations in δ^{13}C and δ^{15}N signatures of A) *Trochosa ruricola* B*)* *Harpalus rufipes* depending on the sampling year.

In summary, managed grasslands represent disturbed ecosystems affected by low disturbances throughout the year, maintaining the high number of species adapted to recover after disturbances. In consequence, the flooding which hit the Jena Experiment was less severe than expected and the soil communities could recover quickly within three months. Moreover, pulse disturbances are parts of all ecosystems and maintaining diversity is important for maintaining resilient ecosystems.

Conclusion

Overall, this thesis gives some insights about the detrimental effects and the rapid response of the soil food web to flooding in grassland ecosystems. My results contribute to understand how the disturbances altered the aboveground-belowground food web. Basal levels of the food web recovered within three weeks. Although the flood almost eradicated Collembola and Acari, they also revovered quickly, but slowlier than microorganisms (three months). The rapid recovery of plants, the sediments deposited after flooding and the biological legacies allowed rapid recovery of density and species richness of all soil organisms studied. The aboveground litter material channel was most disturbed, with effects of the flood propagating through the soil food web up to predators. Three months after the flood microbial functions and microarthropod communities recovered with abundances and species richness resembling that before the flood. However, still microbial nutrient limitation was disturbed and switched from being N limited before the flood to C and P limitation after the flood; further, shifts in species composition, especially in Collembola communities, still were present three months after the flood, reflecting succession processes induced by flooding. Key questions when addressing resilience are those pertaining to the ability of species to recolonize disturbed areas by dispersal from nearby source areas and to the survival of species and structures in the face of disturbances. The spatial configuration of habitats and landscapes is an important determinant of resilience. These issues should receive more attention in the future.

In general, results of this thesis highlight the pronounced resilience of soil organisms against disturbances, with plant diversity in the medium-term fostering the recovery of soil communities. This suggests that the soil communities of European grassland ecosystems are well adapted to disturbances presumably due to the continuous disturbances by management practices. This at least applies to the recovery of soil communities in the medium-term, whereas long-term community responses to large disturbances remain little understood and deserve further investigation.

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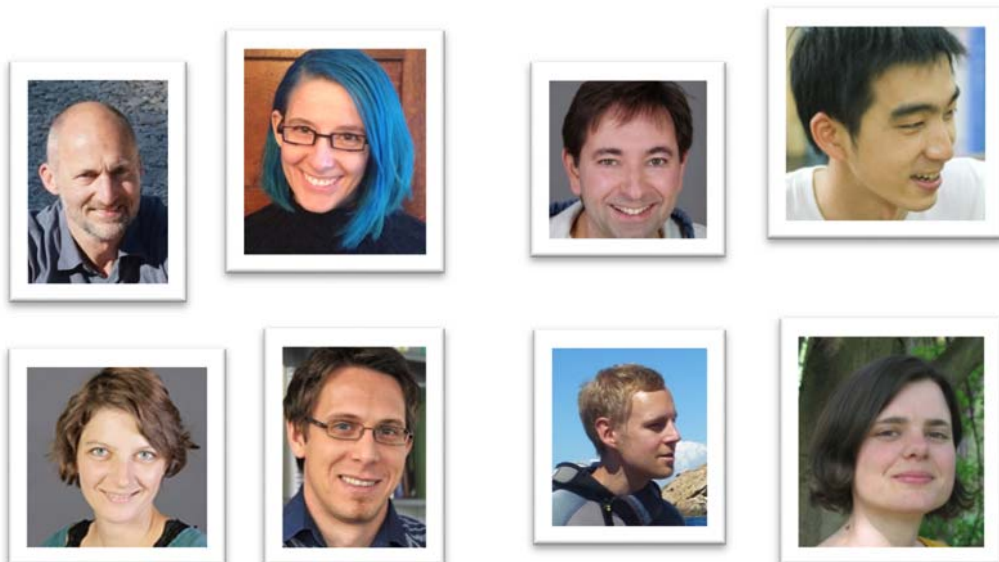
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Submitted

- González Macé O, Scheu S (2017) Response of Collembola and Acari communities to summer flooding in a grassland plant diversity experiment. Submitted in Soil Biology & Biochemistry

Thesis declarations

<u>Declaration of the author's own contribution to manuscripts with multiple authors</u>

Chapter 2 comprise a manuscript that have been published in peer-reviewed journals. Chapters 3 comprises a manuscript that is currently submitted to a peer reviewed Journal.

I am the first author of all chapters; I have developed the main ideas, analysed the data, written the manuscripts and created tables, figures and supplementary materials.

The study design was developed in the framework of the 'Jena Experiment' (Grassland Management) experiment, which forms part of the Cluster of Excellence "Functional Biodiversity Research" (FBR).

I declare that I have written this doctoral thesis independently. All persons contributing to the manuscripts have been named so. All sentences or passages quoted from other people's work have been specifically acknowledged by clear cross-referencing.

 I have not submitted this thesis in any form for another degree at any university or institution.

_____

Odette González Macé
Göttingen, March 2018