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Community composition and functional diversity in riparian wetlands: interactions between hydrology and competition

Gemeenschapssamenstelling en functionele diversiteit in beekdallandschappen: interacties tussen hydrologie en competitie

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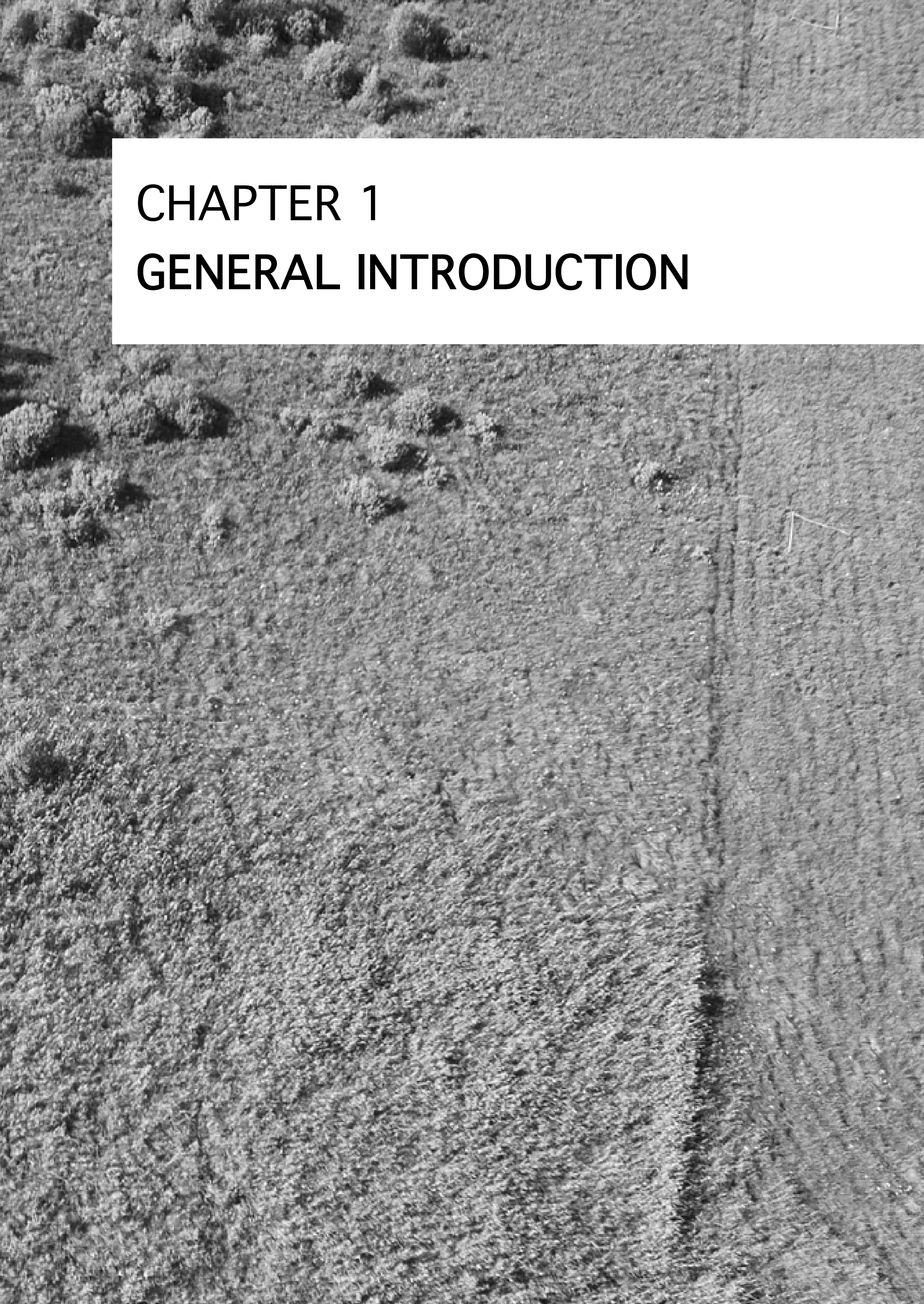
In the spring, at the end of the day, you should smell like dirt.
~Margaret Atwood

CHAPTER 1

GENERAL INTRODUCTION



GENERAL INTRODUCTION

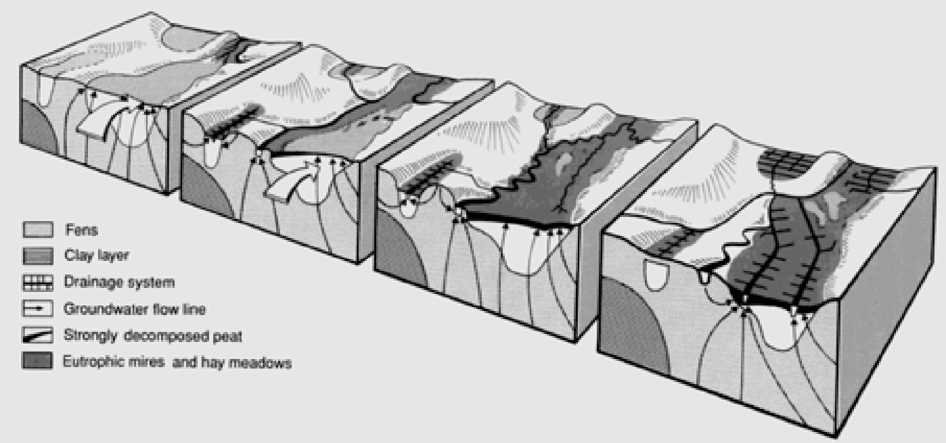
RIPARIAN LANDSCAPES IN EUROPE

Riparian wetlands are among the biologically most productive and diverse ecosystems on earth (Tockner & Stanford 2002). Apart from harbouring a disproportional high biodiversity, natural and semi-natural riparian ecosystems provide several goods and services to humanity, so called ecosystem services. Amongst these, flood mitigation, carbon sequestration and the improvement of water quality through nutrient retention are well known examples (Pfadenhauer & Grootjans 1999, Zedler 2003, Mant & Janes 2006).

Despite their importance for biodiversity and ecosystem services, about half of the global wetland area is estimated to be lost already (Zedler & Kercher 2005). In the European cultural landscape, both natural floodplain forests and semi-natural grasslands are nowadays among the most threatened ecosystems (Tockner & Stanford 2002). Estimates from the United Kingdom indicate that 95-98% of species-rich hay meadows, present before 1940, have been lost (Garcia 1992). Unfortunately, these numbers reflect the situation for other northwest European countries as well (e.g. Muller *et al.* 1998, Grootjans *et al.* 2002).

Although the first human impact on the riparian landscape in northwest Europe already dates back 15000 years, the effects were initially limited (Higler 1993). Grootjans & van Diggelen (1995) and Grootjans *et al.* (2002) provide an extensive summary on the changes in time, due to altered agricultural practices, of the brook valley landscape in northwest Europe (Fig. 1). About 9000-7000 BP, the first agricultural invasion of brook valleys took place, followed by a second around 4600 BP (Grootjans *et al.* 2002, Poschlod *et al.* 2005). This first land use mainly consisted of forest grazing and the growth of arable crops in a shifting cultivation system. From medieval times onwards, land use changed at a larger scale and speed. The degradation and destruction of primeval forests continued, while large oligotrophic bogs, mesotrophic fens and eutrophic reed swamps were drained and reclaimed (Grootjans *et al.* 2002). The once natural landscape had changed into an open, semi-natural landscape where primeval forests and natural mires were gradually replaced by heathland and semi-natural hay and fen meadows. Nevertheless, many species of the original natural landscape persisted.

From the second half of the 19th century, this semi-natural system transformed into a predominantly cultural landscape due to severe land use changes throughout Western Europe (Bakker & Olff 1995, Grootjans *et al.* 2002, Poschlod *et al.* 2005, Jensen *et al.* 2006). In this period, large surfaces of riverine wetlands were converted to arable land through increased investments in drainage works and the application of mineral fertilizers (Fojt & Harding 1995). Together with land use changes in the infiltration areas, this resulted in peat subsidence, increased mineralization, eutrophication, acidification of the top soil, etc (Grootjans *et al.* 1996, Wassen *et al.* 1996, Lamers *et al.* 1998, Olde Venterink *et al.* 2002, van Diggelen *et al.* 2006). From the mid 20th century onwards, changes in policies and agricultural markets caused a further degradation of the remnants of the semi-natural landscape (Poschlod *et al.* 2005, Küster & Keenleyside 2009). The area occupied by nutrient-poor and species-rich habitats, such as wet grasslands, further declined as they were



↑ **Fig 1.** Changes in time in a brook valley landscape due to shifts in agricultural practices. First, natural mires, such as bogs and fens were transformed into semi-natural fen meadows. Subsequently, intensive drainage and fertilisation led to severe ecological degradation (adapted from Grootjans & van Diggelen 1995).

either converted into intensively used agricultural systems, were afforested or abandoned (Bakker & Berendse 1999).

In Europe, land abandonment mainly occurred on qualitatively bad soils like peaty or poor moraine soils (Küster & Keenleyside 2009). This large scale land-use change led to secondary succession in a wide range of vegetation types. In fens and fen meadows, abandonment often results in a fast, initial development towards fallows with tall herbs and tussock-forming sedges, and eventually *Salix* or *Alnus* thickets. This succession may lead to decreased species richness, a replacement of habitat specialist by more generalist species and an increased productivity (Fossati & Patou 1989, Jensen & Schrautzer 1999, Billeter & Diemer 2000, Diemer *et al.* 2001, Peintinger & Bergamini 2006).

To preserve the remaining species-rich wetlands, nature conservationists started to protect and restore these areas during the 1950s and 1960s (Bakker & Olff 1995, Grootjans & van Diggelen 2009). Despite these efforts, many fen species still face extinction. Estimates for the Netherlands indicate that at least 50% of all groundwater dependent ecosystems have been moderately to strongly affected by hydrological changes (Runhaar *et al.* 1996). Despite the vast increase of our knowledge on the limits and feasibility of wetland restoration (e.g. Wheeler 1995, Bakker & Berendse 1999, Pfadenhauer & Grootjans 1999, Klötzli & Grootjans 2001, Jensen *et al.* 2006, Klimkowska *et al.* 2007), knowledge gaps on the ecology and functioning of riparian systems (e.g. driving factors of community assembly, consequences of abandonment on functional composition and nutrient cycling, interactions between above- and belowground biota, …) still remain.

COMMUNITY COMPOSITION IN RIPARIAN LANDSCAPES

Undisturbed lowland brook valleys are characterised by a vegetation gradient perpendicular to the river (Grootjans 1980, Pałczyński 1984, Succow 1988). Along the valleys slopes, nutrient-poor heathland communities were present, whereas alder woods dominated at the valley margin. Floodplains with highly productive reed and large sedge communities were present near the river. In between, low productive fen vegetation with a high abundance of small sedges and brown mosses, persisted (Grootjans 1980, Pałczyński 1984).

Most northwest European lowland valleys once supported such a complex mire system with its characteristic zonation (Wassen *et al.* 1990, van Diggelen 1998). Unfortunately, such natural gradients have now become very rare in Western Europe, due to the large scale exploitation of brook valleys. This already started in medieval ages, with alder brooks being cut and mires drained. As long as drainage remained superficial, the original vegetation was replaced by semi-natural communities. Fertile sites, often close to the river, harboured eutrophic meadows and tall herb communities, while mesotrophic litter meadows and fen meadows developed at less productive sites (Kotowski & van Diggelen 2004).

DRIVERS OF COMMUNITY COMPOSITION

Hydrological gradients have traditionally been considered as one of the main driving factors for species distribution in lowland riparian systems (e.g. Grootjans 1980, Pałczyński 1984, Succow 1988, Wassen *et al.* 1990, Silvertown *et al.* 1999). In a pristine riparian landscape, four water sources can be discerned: lateral-flowing groundwater, discharging groundwater from deeper aquifers, surface water and rainwater (Grootjans 1980, Succow 1988, van Diggelen 1998). Perpendicular to the river, the relative influence of these sources changes, resulting in a zonation of hydrological conditions and thus a discernable vegetation gradient: i) a floodplain zone near the river with high water level fluctuations and the input of nutrient-rich surface water, ii) a broad mesotrophic transition zone with an assumed lateral groundwater movement and iii) eutrophic spring mires near the valley margin (Succow 1988, van Diggelen 1998). This hydrological gradient controls other environmental factors in a complex way (Kotowski *et al.* 2001). First of all, water levels affect peat mineralization rates, leading to a nutrient availability gradient (Koerselman *et al.* 1993, Okruszko 1995). Next, the hydrological regime determines soil oxygen levels and thus the redox potential, which influences many biochemical processes (de Mars & Wassen 1999, Lamers *et al.* 2006). Finally, chemical water composition may interact with effects of water level fluctuation. For instance, water table fluctuations interact with groundwater quality in driving phosphate availability (Lucassen *et al.* 2005, Smolders *et al.* 2006).

Yet, productivity gradients do not always coincide well with the hydrological gradient. Especially in areas where human management has interfered with natural patterns, this relation is disturbed (Bridgham *et al.* 1996, Kotowski & van Diggelen 2004). Kotowski *et al.* (2006) showed that productivity gradients and their influence on competition intensity are probably the prime factor structuring vegetation zonation in lowland riparian fens. Seemingly, species distribution in riparian landscapes is jointly controlled by the hydrological regime and competition (Weiher & Keddy 1995, Lenssen *et al.* 2003, Kotowski & van Diggelen 2004, Kotowski *et al.* 2006). This relationship has however mainly been studied at the level of individual species (Kotowski *et al.* 2001, Lenssen *et al.* 2003) and their outcomes can hardly be extrapolated to the community level. Studies which assess the impact of competition and waterlogging on species interactions in multispecies communities are therefore urgently needed (cf. Stockey & Hunt 1994, Weiher & Keddy 1995, Lenssen *et al.* 1999).

Despite the evidence that the composition of both the plant and soil microbial communities is affected by hydrology, few studies have focused on plant-microbial interactions in freshwater wetland soils (Hartman *et al.* 2008) or investigated soil moisture content as a common driving factor for both the soil microbial and plant community composition (Balasooriya *et al.* 2008, Eskelinen *et al.* 2009, Yu & Ehrenfeld 2010). Nevertheless, the plant and soil microbial community composition are intrinsically linked (Wardle *et al.* 2004) with both positive and negative feedbacks (Wardle 2002, Reynolds *et al.* 2003, Wardle *et al.* 2004, van der Heijden *et al.* 2008, Kardol & Wardle 2010)

In semi-natural landscapes, traditional grasslands (often called "semi-natural") are of high conservation value as they support many Red List species (Küster & Keenleyside 2009). Unlike the term "natural area" suggests, most fens and floodplains can not be preserved without regular human intervention (van Diggelen *et al.* 2006) and hence depend on non-intensive management like mowing or extensive grazing (Bakker 1989). These management practices add-on or interfere with the effects of hydrology and competition in structuring wetland plant communities, and thus form an extra driving factor of community composition which needs to be taken into account.

PLANT TRAITS

To understand the processes which occur during community assembly, wetland degradation or secondary succession, species-traits can be used instead of just species identity. Traits often reflect a species adaptation to its environment (Westoby *et al.* 2002, McGill *et al.* 2006, Menezes *et al.* 2010). Hence, a trait-based approach not only allows a comparison of the same process in different vegetation types (e.g. Díaz *et al.* 2001, Lavorel & Garnier 2002, Kahmen & Poschlod 2008, Purschke *et al.* 2012), but also provides insight into the mechanisms responsible for such patterns (Kahmen & Poschlod 2004). As such, plant traits can also be used as predictors of performance in ecological restoration (Pywell *et al.* 2003).

The availability of different data bases with life-history traits of the northwest European flora (e.g. Ellenberg *et al.* 1992, BioBase: CBS 2003, BiolFlor: Kuhn *et al.* 2004, CloPla: Klimešová & Klimeš 2006, LEDA: Kleyer *et al.* 2008, SID: Liu *et al.* 2008), together with the development of several multivariate techniques (e.g. RLQ: Dolédec *et al.* 1996, Concordance analysis: Lafosse & Hanafi 1997, Fourth Corner method: Legendre *et al.* 1997, Dray & Legendre 2008), allowed for the linkage between species traits and environmental characteristics, further enhancing our understanding of ecosystem functioning.

A plant ecological strategy sensu Grime (1979, 2001) involves a suite of different plant traits, embedded in a web of direct and indirect causal relationships, which may vary in relation to environmental conditions (Vile *et al.* 2006). Within wetlands, tussock development by sedges is such a plant ecological strategy. Tussock growth can be one of the possible structural changes to evade the detrimental effects related to the hypoxic or anoxic status of flooded and waterlogged soils (Colmer & Voesenek 2009). Tussock forming plants create their own aerobic environment above their waterlogged surroundings and as such engineer their environment. As many organisms strictly depend on oxygen for their survival, the impact of ecosystem engineers on soil oxygen conditions potentially affects the fitness of other species (Caraco *et al.* 2006).

NUTRIENT CYCLING IN RIPARIAN LANDSCAPES

Riparian wetlands represent the link between terrestrial and aquatic environments and interact strongly with river biogeochemistry. Furthermore, they act as large buffers between upstream ecosystems and rivers and estuaries. The importance of wetlands in nutrient cycles (mainly N and P) as sinks and processors has been studied extensively (e.g. Güsewell & Koerselman 2002, Gribsholt *et* *al.* 2005), highlighting the role of riparian wetlands in reducing excessive N and P inputs to aquatic ecosystems (e.g. Hattermann *et al.* 2004, Olde Venterink *et al.* 2006). Yet, the role of wetlands in Si cycling has remained largely unknown, which can mainly be attributed to the recent recognition of the importance of terrestrial ecosystems (Conley 2002), and wetlands in particular (Struyf *et al.* 2006, Struyf & Conley 2009), in controlling terrestrial Si fluxes. The solubility of biogenic amorphous silica (BSi) stored in soils as plant phytoliths and diatoms, is several orders of magnitude higher than that of mineral silicates (Farmer *et al.* 2005). Hence, the recycling of BSi exhibits an important control on terrestrial dissolved silica (DSi) export (e.g. Derry *et al.* 2005, Fulweiler & Nixon 2005, Sommer *et al.* 2006). DSi mobilization in the terrestrial environment is the primary silica source for the aquatic, and eventually coastal and oceanic, environments (Van Cappellen 2003), where it is an essential nutrient for diatoms (*Bacillarophyceae*). The availability of DSi relative to other nutrients can influence the occurrence of harmful algal blooms (e.g. Smayda 1997).

The high abundance of grasses and sedges in wetlands (i.e. silica rich biomass), together with the frequent occurrence of diatoms and sponges, provides the potential for the accumulation of BSi-rich soils (Clarke 2003, Struyf *et al.* 2005). Struyf and Conley (2009) hypothesized that a trade-off between BSi storage in wetland soils and the export of DSi from wetlands depends on hydrological and vegetation characteristics. However, little research has actually focused on the factors controlling accumulation and recycling of BSi in wetlands that could verify this hypothesis.

AIM OF THIS WORK

Within the framework of this study, we aimed to enhance the understanding of the factors governing community composition and functional diversity in riverine wetlands. Here, we mainly focused on the effects of hydrology and competition, and how management interferes. Next, the effects of hydrology and management on Si cycling are explored.

OUTLINE

In **Chapter 2**, the relationship between habitat and competition filtering in community (re)assembly in river floodplains was tested. Here, flooding changes both habitat stress and competition on the one hand and acts as an effective seed dispersal vector on the other hand. A mesocosm experiment on early community assembly was conducted, starting with a pool of 34 floodplain species covering a wetness gradient. Seed mixtures were sown in a full factorial design with water level, canopy and mowing as controlling factors.

In **Chapter 3**, the soil microbial community composition of the mesocosm experiment described in Chapter 2 was analysed using PLFAs. Vegetation and soil microbial community composition are known to be intrinsically linked. Yet, only few studies investigated hydrology as a common driving factor for structuring both communities. In this chapter the effect of the experimental parameters on the composition of both the microbial and plant communities was determined and potential differences in effects were compared between both communities.

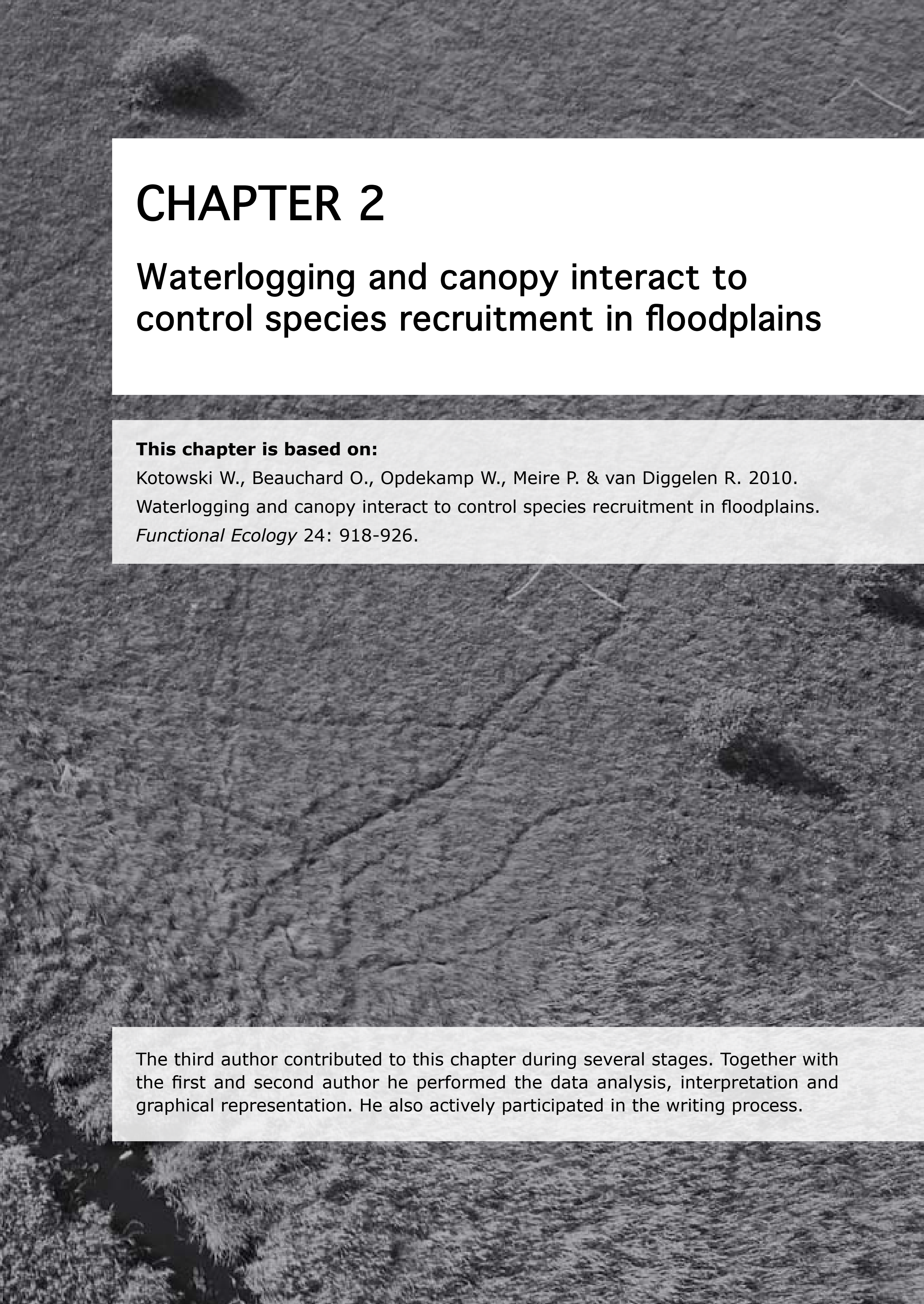
In **Chapter 4**, the interactions between mowing cessation and hydrology in determining species and trait distribution were examined using a field study in the Upper course of the Biebrza National Park (Poland). Along four transects, 15 sites were selected, with plots in mown and abandoned parcels.

In **Chapter 5**, the role of hydrological and vegetation characteristics in controlling BSi storage and DSi export from a riparian meadow system was explored. Despite the known importance of riparian wetlands in other nutrient cycles (N and P) and the potentially profound influence of riparian landscapes on terrestrial silica transport and storage, little research focused on the factors controlling accumulation and recycling of BSi in riparian wetlands.

In **Chapter 6**, tussock formation, a common growth form observed in many sedge and grass species, was examined as one of the possible structural changes to evade the detrimental effects caused by waterlogging. Furthermore, through the creation of aerated conditions these plants potentially may have considerable effects on the fitness of other species as many organisms depend strictly on oxygen for their survival. Next, their role as ecosystem engineers in riparian landscapes is discussed.

In **Chapter 7**, BSi storage and cycling within these tussocks was investigated. Despite its beneficial role for many graminoid species, little is known about Si availability within tussocks. Next, the consequences of land abandonment, through tussock formation, on BSi storage in riparian landscapes were elaborated.

In **Chapter 8**, the results presented in the previous chapters are discussed in a broader context and the implications for nature conservation are explored.



CHAPTER 2

Waterlogging and canopy interact to control species recruitment in floodplains

This chapter is based on:
Kotowski W., Beauchard O., Opdekamp W., Meire P. & van Diggelen R. 2010. Waterlogging and canopy interact to control species recruitment in floodplains. *Functional Ecology* 24: 918-926.

The third author contributed to this chapter during several stages. Together with the first and second author he performed the data analysis, interpretation and graphical representation. He also actively participated in the writing process.

ABSTRACT

The extent to which seedling recruitment contributes to local functional diversity depends on the environmental filters operating in a plant community. Classical community assembly models assume that habitat constraints and competition act like hierarchical filters with habitat filtering as the dominant one. Alternative models assume a synergic interaction since responses to environmental stress and competition may impose physiological trade-offs in plants.

River floodplains are an ideal system to test the relationship between habitat and competition filtering in community (re)assembly, as flooding causes changes in both habitat stress (waterlogging, resulting in anoxia and toxicity) and competition (dieback of vegetation) on one hand and acts as an effective seed dispersal vector on the other hand.

We conducted a mesocosm experiment on early community assembly from a pool of 34 floodplain species covering a wetness gradient. Seed mixtures were sown in a full factorial design with water level, canopy and mowing as controlling factors. We measured the biomass of all species after one growing season and determined germination and seedling growth traits, both outside (response to waterlogging/no waterlogging) and in a growth-chamber (response to light/darkness).

Species recruitment was analysed in relation to the controlling factors and measured functional traits using co-inertia analysis. Furthermore we analysed the effects of the controlling factors on several aspects of functional diversity.

There was no establishment in grass sward, unless mowing was applied. Species-rich communities only developed when germination and early establishment phases occurred on waterlogged bare soil. High water level did not suppress establishment but reduced the total biomass and lowered inter-specific competition. The effect of mowing on species richness depended upon the interplay between waterlogging and canopy.

Establishment success under canopy required seedling strategies to tolerate shade. The elimination of typical wetland specialists from oxic mesocosms was clearly an effect of their poorer and/or slower germination and lower competitive abilities in comparison to non-wetland plants, leading to their disappearance in this low-stress environment.

Our results indicate that single stress factors can enhance species richness and functional diversity through limiting competition but a synergic interaction of different stresses can lead to reduced richness.

1. INTRODUCTION

The extent to which seedling recruitment contributes to local species richness and functional diversity depends on biotic and abiotic filters operating in a plant community (Díaz *et al.* 1998). Although these two groups of factors, also known as 'competition' and 'habitat filters', normally operate simultaneously, their relative impact may differ from situation to situation. Vegetation ecologists agree on the central role of competition in community assembly under physically optimal conditions and high resource availability, e.g. in mesic grasslands or old fields (e.g. Grime 1979, Walker & Chapin 1986, Tilman 1988), and the dominance of habitat filtering in more extreme habitats, such as deserts or salt marshes (Grime 1979). However, much more uncertainty exists regarding the relative importance of competition and habitat filtering when stress and resource gradients coincide with each other. This question is of particular interest in dynamic ecosystems subjected to frequent disturbance and high rates of seed immigration, where community re-assembly is a frequent process structuring vegetation patterns (Myers & Harms 2009). River floodplains form a typical example of such habitats, where regular inundations result in: i) frequent changes in the level of soil anoxia, a major habitat constraint, ii) large temporal and spatial differences in competition intensity, due to nutrient enrichment by floodwater and temporal die back of submerged vegetation and iii) relatively unconstrained dispersal of seeds with floodwater (Olde Venterink *et al.* 2006, Gerard *et al.* 2008, Kyle & Leishman 2009).

Species distribution in riparian landscapes has been shown to be jointly controlled by water level and competition (Weiher & Keddy 1995, Lenssen *et al.* 2003, Kotowski & van Diggelen 2004, Kotowski *et al.* 2006), but the relationship between these two factors has mainly been studied at the level of individual species (Kotowski *et al.* 2001, Lenssen *et al.* 2003) and these studies seem unsuitable to draw conclusions at the community level. Therefore studies which assess the impact of competition and waterlogging on species interactions in multi-species communities are needed (cf. Stockey & Hunt 1994, Weiher & Keddy 1995, Lenssen *et al.* 1999), but these are, to our knowledge, entirely lacking.

Following Lenssen *et al.* (2003), we identify three possible types of relationship between competition and habitat filtering: i) hierarchy (one factor highly dominates the other one), ii) synergy (the joint effect is larger than the simple sum of the effects of the single factors) and iii) inhibition (one factor reduces the effect of the other one).

Lenssen *et al.* (1999) and Lenssen *et al.* (2003) found that anoxia-intolerant species strongly responded to shading when growing in dry soil, whereas they were highly suppressed by oxygen-stress under waterlogged conditions and shading did not cause any further important effects. This is in accordance with the existing hierarchical model of environmental filters, where habitat filtering is considered to be dominant over competition (Zobel *et al.* 1998). Only species that can tolerate reduced waterlogged conditions would enter into competition in wetlands.

The presumed trade-off between the simultaneous use of limited below- and above-ground resources (Smith & Huston 1989) suggests a synergic relation between waterlogging and light stress. Under low light availability the root/shoot ratio decreases (Kotowski *et al.* 2001), whereas waterlogging can induce -apart from physiological changes- morphologic adaptations such as additional investments into roots (Visser *et al.* 2000), the development of adventitious shallow roots or aerenchyma (Jackson *et al.* 2009), etc. This trade-off would suggest that plants are more sensitive to competition for light when growing in water-saturated, anoxic soils.

Lenssen *et al.* (2003) also suggested an inhibition between waterlogging and competition for light. Since the growth rate of plants decreases under reduced conditions, even in typical wetland dominants (Brix & Sorrell 1996), light competition may be less intense on waterlogged soils than on aerated ones.

Floodplains which are often eutrophic, can support very species-poor as well as moderately species-rich communities, both on waterlogged as well as aerated soils. Thus, understanding the mechanisms that mediate floodplain botanical diversity, is important; not only for the advancement of our knowledge concerning community functioning, but also to optimise strategies to maintain and restore riparian ecosystems. Hence, the objective of the present study is to understand the relative roles of waterlogging, vegetation canopy, and the nature of their interaction, in filtering a floodplain species pool, and thus their potential control on local botanical diversity. We approach this question by a multispecies mesocosm experiment and interpret the results in terms of functional traits (McGill *et al.* 2006, Haddad *et al.* 2008, Villéger *et al.* 2008). Habitat and competition filters are believed to affect functional diversity (FD) differently: habitat filtering is assumed to lead to convergence in traits (only species that can tolerate certain levels of environmental stress pass through), whereas competition would lead to divergence (Cornwell *et al.* 2006, Grime 2006). We used the framework of Mason *et al.* (2005) and Villéger *et al.* (2008) that highlights three different facets of functional diversity. We interpret functional richness (FRic) as a measure of the total amount of filtering, functional evenness (FEve) as a measure of niche overlap and functional divergence (FDiv) as a measure of functional heterogeneity.

Our questions are as follows: i) What is the relative impact of canopy and waterlogging on species recruitment? ii) What type of relationship exists between these two factors? iii) Does a temporal relief of above-ground competition by mowing in the middle of a growth period enhance recruitment?

2. METHODS

2.1 Selected species

We selected 34 species that occur frequently in floodplains of temperate Europe and represent the whole moisture gradient from mesic meadows to reed beds and swamps (Table 1). We deliberately selected species with different seed size within each moisture category. Seeds were obtained from commercial seed suppliers (Ecoflora, Halle, Belgium; De Bolderik, Wervershoof, the Netherlands; De Morgenster-zaden, Bussum, the Netherlands) as 'ready-to-use'.

↓ **Table 1.** Species selected for the experiments, F = Ellenberg moisture indicator (Ellenberg *et al.* 1992), SM = mass of 1000 seeds (amount introduced to each mesocosm); names follow Tutin *et al.* (2001).

mesic habitat species	F	SM (g)	**wetland species**	F	SM (g)
Achillea millefolium	4	0.17	*Carex ovalis*	7	0.49
Daucus carota	4	0.87	*Cirsium oleraceum*	7	1.90
Galium verum	4	0.54	*Eupatorium cannabinum*	7	0.22
Leucanthemum vulgare	4	0.43	*Geum rivale*	7	1.21
Angelica sylvestris	5	2.46	*Cirsium palustre*	8	1.11
Centaurea jacea	5	2.33	*Epilobium hirsutum*	8	0.11
Heracleum sphondylium	5	7.35	*Filipendula ulmaria*	8	0.60
Leontodon autumnalis	5	0.80	*Lythrum salicaria*	8	0.06
Lolium perenne	5	0.90	*Myosotis palustris*	8	0.28
Plantago lanceolata	5	2.63	*Phalaris arundinacea*	8	1.02
Poa pratensis	5	0.31	*Senecio aquaticus*	8	0.24
Prunella vulgaris	5	0.86	*Valeriana officinalis*	8	0.72
Rumex acetosa	5	0.81	*Carex acuta*	9	0.90
Alopecurus pratensis	6	0.71	*Lychnis flos-cuculi*	9	0.16
Ranunculus acris	6	1.79	*Lycopus europaeus*	9	0.27
Urtica dioica	6	0.16	*Peucedanum palustre*	9	1.35
Veronica longifolia	6	0.07	*Typha latifolia*	10	0.04

2.2 Screening for functional traits

Germination in relation to light

Species were tested for their germination capacity under light and dark conditions in a growth chamber (21 °C, photoperiod of 16h light/8h dark). 50 or 100 seeds per species were sown on top of sterile compost soil in transparent plastic boxes (70 x 100 x 30 mm) with five replicates. Soil moisture was checked every few days and demineralised water was added if necessary. Photosynthetically Active Radiation (PAR) (AccuPAR LP-80 Ceptometer, Decagon Devices Inc., Pullman, USA) in the light treatment was 130 (SD = 15) μmol m^{-2} s^{-1}, whereas the dark treatment consisted of boxes wrapped in two layers of aluminium foil. Sowing and counting seedlings was carried out under light levels not exceeding 2 μmol m^{-2} s^{-1}. Germinated seeds were counted and seedlings were removed every 3-4 days. We used germination percentage in light (GP), time of 50% germination (T50) and the ratio between light and dark germination percentage (DGR) as functional traits for further analyses (Table 2).

Germination in relation to wetness

We tested the germination ability of species within the mesocosm set-up (see below), both in wet and dry treatment, using 10 cm margins on the northern edge of containers (to avoid shading from the edge). We used 4 replicates per species and treatment. Each replicate was installed in a different container and consisted of 50-200 seeds (depending on seed size) sown in a 10x10 cm transparent plastic box with removed bottom, inserted into the soil for 2 cm. Seedlings were counted and removed after 20 and 40 days. We expressed the response to wetness as the ratio between germination in wet and dry mesocosms (WGR; see Table 2).

Seedling growth parameters

Seeds that had germinated in the climate chamber germination experiment were transplanted to plastic pots of 6cm-diameter filled with compost soil, as soon as possible after germination. We followed procedures described by Hunt *et al.* (2002) for seedling growth, using 7 days-old and 22 days-old seedlings for calculating the relative growth rate and biomass allocation ratios (Table 2).

Instead of dry weight, we used fresh weight due to the very small size of some seedlings. During growth, plants were watered from below with a nutrient solution containing 0.042-0.021-0.042 g N-P-K dm^{-3} respectively. The height of 7-days old seedlings was measured before harvesting, in case of grasses and sedges we measured the distance from soil surface to the tip of the longest leaf. Leaf area was measured on digital photographs of leaves using ImageJ (Rasband 1997-2008).

↓ **Table 2.** Traits selected for analysis

Germination traits	
SW	seed weight of 2 x 100 seeds (mg)
GP	total germination percentage in full light (%)
DGR	dark germination ratio (dark germination/light germination) (dimensionless)
T50	time of 50% germination in light (days)
WGR	wet germination ratio (germination in wet mesocosms/germination in dry mesocosms) (dimensionless)

Seedling Traits	
H7	average height of seedlings at 7th day from germination (mm)
LA7	mean leaf area of seedlings at 7th day (mm^2)
LWR7	mean leaf weight ratio at 7th day (leaves and cotyledons) (dimensionless)
LWR	mean leaf weight ratio, increase between the 7th and 22nd day (dimensionless)
LAR7	mean leaf area ratio, i.e. mean quotient of the total leaf area per plant and the total weight per plant at 7th day (mm^2 mg^{-1})
LAR	mean leaf area ratio, i.e. mean quotient of the total leaf area per plant and the total weight per plant; increase between the 7th and 22nd day (mm^2 mg^{-1})
AGR	mean actual growth rate of seedlings between the 7th and 22nd day (mg day^{-1})
RGR	mean relative growth rate of seedlings between the 7th and 22nd day (dimensionless)
SLA	mean specific leaf area, i.e. mean quotient of the total leaf area per plant and the total leaf weight per plant; increase between the 7th and 22nd day (mm^2 mg^{-1})

2.3 Mesocosm experiment

We established a mesocosm experiment in open air to study the impact of waterlogging, canopy-shading and a temporal alleviation of light stress through mowing on the first phases of establishment of herbaceous plant communities. The mesocosms consisted of PVC containers (111 x 91 x 61 cm height), filled with a drainage substrate for 25 cm (Argex clay aggregate, Argex NV, Zwijndrecht, Belgium) separated by geo-textile from a 25 cm cover of a soil mixture (3:1 volumetric parts of fen peat and alluvial clay). To ensure eutrophic conditions throughout the experiment, an initial amount of 168g N, 192g P, 216g K and 24g Mg (PG-mix Floranid, COMPO Benelux nv, Deinze, Belgium) per m^3 soil, and a slow release fertilizer (6 months release time; Osmocote, SCOTTS Professional, Sint-Niklaas, Belgium) containing 300g N, 180g P and 180g K per m^3 soil were added. These values are at the upper limit of annual nutrient input in European floodplains (Pinay *et al.* 1995, Olde Venterink *et al.* 2006). Eighty mesocosms were positioned in a block of eight rows. Water was applied through sprinklers installed 30 cm above each container once (spring, autumn) or twice (summer) a day.

We applied the following treatments in a full-factorial design with 10 replicates for each treatment combination (given abbreviations are used later in the text):

- C/nC – germination under canopy of *Poa pratensis*, *Lolium perenne* and *Alopecurus pratensis* vs. germination on bare ground; grasses were pre-grown for 6 weeks and cut at 10 cm height at the sowing date (the species mixture ensured that canopy persisted under both dry and wet conditions);
- W/nW – waterlogged treatment with water level maintained 5 cm below soil surface vs. non-waterlogged (aerated) treatment with water percolation through soil profile and air penetration from below;
- M/nM – mowing of vegetation at 2cm height applied at 6th week of experiment (end of June 2006) vs. no mowing.

Thirty-four species were sown into the mesocosms on May 15th 2006 with a density of c.1000 seeds $species^{-1}$ m^{-2}. Poa, Alopecurus and Lolium were used as matrix vegetation in the C treatment but were also part of the species pool introduced to all containers. The number of seeds was determined by weight, after establishing the weight of 100 seeds with an accuracy of 0.001 g. The total number of seeds, 37000 m-2, was similar to the average seed production on wet meadows (Klimkowska *et al.* 2009).

During the experiment, an AccuPAR LP-80 Ceptometer (Decagon Devices Inc., Pullman, USA) was used to measure light attenuation in all mesocosms at 0 and 10 cm above ground surface.

During the last week of September 2006, above-ground biomass of all mesocosms was harvested and sorted to species level (except for Poa, Lolium and Alopecurus, which were grouped together). Throughout the experiment, care was taken to remove incidental individuals of non-introduced species, however if some of them remained in the communities, they were separated during harvesting. Dry mass was measured after 72 hours of drying at 70 °C.

2.4 Data analysis

Traits

The measured values allowed for the calculation of a number of traits characterising germination and establishment. We selected those with a clear ecological meaning avoiding strongly correlated pairs of traits (cf. Lepš *et al.* 2006) (Table 2).

Vegetation indices

Treatment effects on the total biomass and the biomass of the tested species of the September harvest, species richness and Pielou’s Evenness index (Beisel *et al.* 2003) (excluding the three matrix grass-species) were analysed with factorial ANOVA.

Species community pattern and relationship with biological traits

The biomasses of all tested species were arranged in an 80 containers × 28 species table to explore species community patterns by means of a centred PCA, after log (x + 1) data transformation. Subsequently a PCA on Instrumental Variables (PCAIV: Sabatier *et al.* 1989, Lebreton *et al.* 1991) was used to compare the influence of the three factors canopy, waterlogging and mowing in shaping the community structure. The statistical significance of the effects was tested based on 9999 random permutations of the rows of the containers × species table.

The relationship between the species × traits table (trait-columns as species ability scores) and the transposed species × treatments table (treatment-columns as species responses) was tested using a Co-Inertia Analysis (CIA: Dray *et al.* 2003). CIA constructs axes encompassing only the common ecological information between both patterns, and the statistical significance of the link was tested based on 9999 random permutations of the rows of the two tables.

Functional diversity of communities

Functional richness (FRic), functional evenness (FEve) and functional divergence (FDiv) were quantified by applying the framework of Villéger *et al.* (2008). These indices structurally summarize different aspects of functional diversity which can be considered, a priori, as independent. Instead of using all traits to calculate these indices for the experimental communities (which could be affected by redundancy) or conducting a preliminary selection (which could be arbitrary), we calculated these indices based on the scores of the species obtained from the first two axes in CIA. We thus considered two synthetic and independent functional traits as suggested by Villéger *et al.* (2008); moreover, the scores of species on the co-inertia axes ensure the use of functional traits significantly related to environmental conditions.

Poa, *Alopecurus* and *Lolium* were considered as matrix vegetation and excluded from the above mentioned procedure in all mesocosms. Also, the CnM treatment, due to zero establishment, was excluded from this analysis.

Treatment effects for FRic, FDiv and FEve were analysed using factorial ANOVA.

Computations and associated graphical representations were implemented using the ade4 package (Dray & Dufour 2007) available in R freeware (R Development Core Team 2009).

3. RESULTS

3.1 Plant functional traits

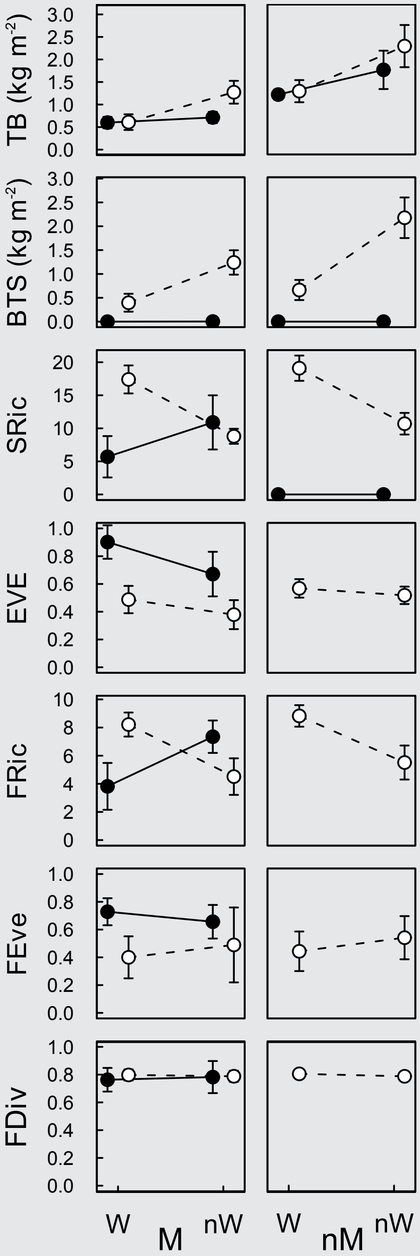
Values of all measured traits are given in Appendix A.

Germination ability ranged from 0 to 95%. Of 34 sown species, 5 had a very low germination rate in the climate chamber: *Carex acuta* (0% germination after 40 days), *Heracleum* (0%), *Angelica* (1.2%), *Peucedanum* (1.6%), *Typha* (1.6%). *Heracleum* and *Carex acuta* did hardly germinate in the outdoor experiment either. Germination rates in the climate chamber experiment (light treatment) and outdoor experiment (moist soil) were highly correlated ($r = 0.68$; $p < 0.001$), with generally a higher germination percentage in the climate chamber.

Waterlogging had a significant effect on the germination rate of seven species: four germinated better on water-saturated soil: *Peucedanum*, *Myosotis*, *Lythrum* and *Typha*. However, only the latter depended completely on water-saturated soil for germination. Three species had a significantly higher germination on aerated soil. Surprisingly, two of these species are related to rather wet habitats (*Angelica sylvestris* and *Cirsium palustre*). *Prunella vulgaris* is the only species of ‘dry’ conditions in this group.

Darkness significantly decreased the germination of 14 species and increased the germination of one species (*Poa*). Only few species were strictly light-dependent for germination: *Carex ovalis*, *Lythrum* and *Lycopus*.

Seedling growth parameters were obtained for 29 species. RGR differed more than twofold between the fastest growing (*Urtica*) and slowest growing species (*Valeriana*). The height of 7 day old seedlings (H7) ranged from 4 mm (*Lychnis*) to 113 mm (*Lolium*). Leaf weight ratio (LWR; range: 0.67-0.93) did not differ much among species; much larger differences were found for the leaf area indices, with ranges of leaf area at the 7th day between 0.10 and 2.81 cm^2 (LA7) and increase in LAR (day 7 – day 22) from 1.39 to 7.39 $mm^2\ mg^{-1}$, SLA between 1.56 to 9.02 $mm^2\ mg^{-1}$.



↑ **Fig 1.** Total dry biomass (TB), dry biomass of tested species (BTS), species richness (SRic), Pielou's eveness (EVE), functional richness (FRic), functional evenness (FEve) and functional divergence (FDiv) in reference to Waterlogging (W), Mowing (M) and Canopy (—●— C; --○-- nC); n = no; shown are means ± SD.

3.2 Mesocosm experiment

Effect on productivity and light interception

Waterlogging reduced the total above-ground biomass production (Fig. 1); this effect was stronger in nC than in C treatment (interaction C × W; Table 3). Moreover, growth rate decreased in W, indicated by more PAR reaching the lower levels of the vegetation (Fig. 2).

Canopy imposed severe shading, preventing more than 99% of PAR to reach the soil surface for most of the growing season. Light availability in nC conditions decreased more gradually with increasing above-ground biomass, with at least 40% of PAR reaching the soil surface 4 weeks after sowing (Fig. 2).

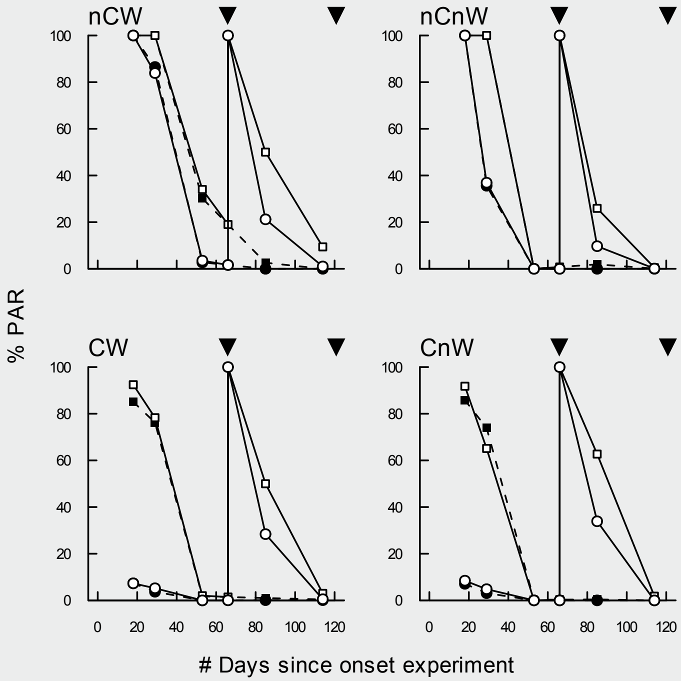
Mowing decreased the total biomass harvested in September (Fig. 1, Table 3) and an increased PAR was observed in all M treatments 3 weeks after mowing but this effect had gone after one month (Fig. 2).

Effects on species establishment

Seedling establishment of all species was drastically suppressed in C treatments, decreasing species number as well as biomass (Fig. 1, Table 3). Although germination was observed here, all seedlings died within a few weeks. This effect was slightly lowered by mowing, resulting in limited establishment of few species in CM (cf. interaction C × M, Table 3). In terms of biomass production this establishment was still negligible compared to the nC treatment (Fig. 1, Table 3), where multi-species plant communities developed (Fig. 1, see Appendix B). Canopy largely increased evenness in the (sub)community of established species (Fig. 1, Table 3).

Waterlogging interacted with canopy and mowing in controlling species richness. Under canopy, where tested species only established after mowing, waterlogging decreased the number of species from 12 (CnWM) to 6 (CWM). The opposite situation was found in the absence of canopy, where nCW mesocosms contained twice as many species as the nCnW.

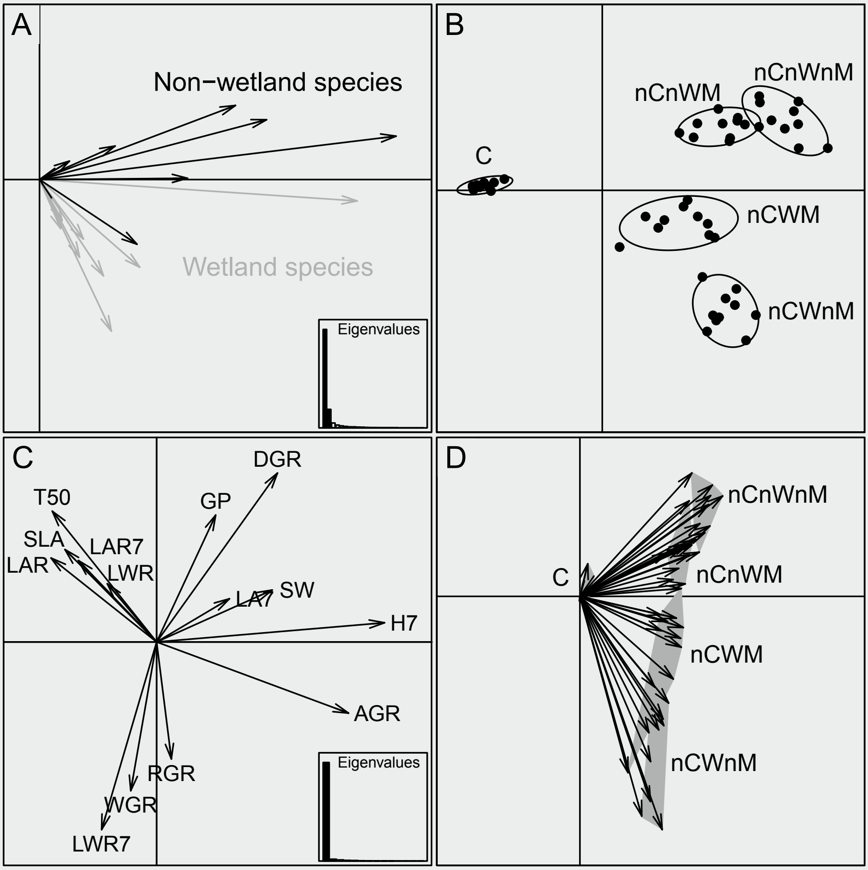
Growth of the established species was largely reduced by waterlogging (Fig. 1, Table 3); this effect was much stronger in C (after mowing) (waterlogging decreased biomass of tested species from 3.0 g m^{-2} to 0.18 g m^{-2}, i.e. by 94%) than in nC treatment (decrease by 66%).



↑ **Fig 2.** Percentage of light reaching the soil surface and 10cm above soil surface in the mesocosms, in reference to treatments. Error bars are not shown for clarity, each point shows the averaged value of 5 or 10 measurements. C = canopy, W = waterlogging, M = mowing; n = no; —●— 0cm nM; --○--0cm M; —■— 10 cm nM; --□--10 cm M. Black triangles indicate mowing dates.

↓ **Table 3.** *F*-values and significance for effects of treatments on community properties (abbreviations in Fig. 1); for EVE, FRic, FDiv and FEve only mown treatments are analysed; df = degrees of freedom; significance: *p < 0.05; **p < 0.01; ***p < 0.001. *F*-values in bold indicate significant effects.

Effect	**Df**	**TB**	**BTS**	**SRic**	**EVE**	**FRic**	**FEve**	**FDiv**
C	1	**51.44*****	**5998.63*****	**596.83*****	**20.23*****	0.65^{ns}	**18.08*****	0.62^{ns}
W	1	**108.29*****	**108.94*****	3.46^{ns}	1.12^{ns}	0.21^{ns}	0.15^{ns}	0.91^{ns}
M	1	**304.78*****	0.13^{ns}	**215.63*****	-	-	-	-
C×W	1	**21.56*****	**19.85*****	**49.24*****	**7.85*****	**27.14*****	**6.69****	0.32^{ns}
C×M	1	0.29^{ns}	**55.49*****	**278.53*****	-	-	-	-
W×M	1	0.02^{ns}	**9.85****	**5.08***	-	-	-	-
C×W×M	1	2.91^{ns}	**8.09****	**8.42****	-	-	-	-



↑ **Fig 3.** Species community pattern and biological responses to treatments. Top: PCA on treatments × species table; **A)** species projections, respectively those for which Ellenberg moisture indicator ranges from 4 to 6 (black) and from 7 to 10 (grey), and eigenvalues diagram (F1, 73.9%; F2, 13.9%); **B)** projections of the containers grouped per treatment. Bottom: Co-inertia analysis between biological traits **(C)** and treatments **(D)**. For abbreviations see Table 2 and Fig. 2.

Species community pattern

The first PCA axis opposes the C mesocosms to those with nC treatments (Fig. 3A & 3B). The conjoined effect of the three controlled factors explained almost the totality of the observed pattern (PCAIV: explained inertia = 90.5%; $p < 0.0001$). The canopy effect was found to be highly significant (explained inertia = 69.8%; $p < 0.0001$), indicating extremely low establishment of species in C treatments, thus engendering a biomass gradient characterized by positive species covariances on the first axis.

The second axis highlights a divergence between species covariances, corresponding to a waterlevel gradient which was found to be significant (explained inertia = 6.7%; $p = 0.0068$), particularly in nC treatments (significant interaction between canopy and waterlogging; explained inertia = 6.3%; $p = 0.0125$). The effect of waterlogging clearly separated wetland species from non-wetland species (as indicated by Ellenberg index).

The mowing effect alone was found to be low and not significant (explained inertia = 2.3%; $p = 0.1549$) and non-significantly interactive neither with canopy nor waterlogging ($p > 0.0500$). Its influence is limited to the conjoined effect where establishment is slightly enhanced in C conditions and where biomass is reduced in nC conditions, particularly in W treatments.

Co-inertia analysis (Fig. 3C & D) revealed a highly significant co-structure between biological traits and species responses ($p < 0.0001$). The first factorial plane encompasses respectively 68.1 and 98.0% of both inertia patterns. The first axis encompasses species' responses to canopy effect, characterized by high scores of seedling height (H7), actual growth rate (AGR), seed weight (SW), and mean leaf area of seedlings at 7th day (LA7) in nC conditions, opposed to slower germination (high T50) and high scores of both LAR at day 7 (LAR7) and increase in LAR between 7th and 22nd day (LAR), SLA and increase in LWR between 7th and 22 day (LWR) in C conditions. On the second CIA axis, W conditions are characterized by high LWR at day 7 (LWR7), good germination in wet mesocosms (WGR) and high RGR, opposed to high ability for dark germination (DGR) and high total germination percentage in light (GP) in nW conditions.

Functional diversity of communities

FRic was largely influenced by the interaction between waterlogging and canopy; waterlogging caused a decrease of this index in C and an increase in nC conditions (Fig. 1, Table 3), whilst neither of these factors caused any significant effect when considered separately. Mowing had no significant effect on FRic.

FEve was increased by the presence of grass canopy during establishment, whilst the effects of waterlogging and mowing were not significant (Fig. 1, Table 3). Significant C × W interaction indicates that waterlogging decreased FEve in nC and increased in C conditions.

FDiv did not respond significantly to any of the factors.

4. DISCUSSION

4.1. The interplay of waterlogging and canopy effects

A major objective of our study was to compare the effects of competition from canopy and waterlogging on the selection from a floodplain species pool. Clearly, canopy presence was a much stronger filtering factor, almost totally disabling establishment, which is probably due to high light attenuation, but possibly an increase in root competition could also play a role. This effect could only be reduced slightly by mowing, which opened the canopy for a short time period.

However, contrary to our expectations, waterlogging alone did not exclude any species from the community and allowed for the highest species richness in the absence of a closed canopy at the moment of seed addition. This effect of waterlogging can be due to different non-exclusive reasons; e.g. waterlogging could act directly through oxygen deficiency and toxicity of reduced S, Fe and Mn (Cronk & Fennessy 2001), seedling growth might be reduced since root growth possibly became more focussed upon the smaller soil volume of the uppermost soil layers. In the non-canopy treatment, water saturation was indeed a stress factor for most of the species but it resulted only in decreased growth rates, not hampering germination or species richness directly. This in its turn led to higher light availability throughout the experiment and presumably lower competition for light between the establishing plants. Hence, almost all species established in the waterlogged non-canopy treatment, whilst a strong competition filtering occurred in the aerated variant.

In contrast to the non-canopy treatment, waterlogging had a negative effect both on species and functional richness in the canopy-mesocosms. This could be explained by increased root competition from grasses, which produced dense shallow roots in the waterlogged treatment. Another explanation can be drawn from the interactive effects of canopy, waterlogging and mowing on light availability. Due to more intense competition between grasses in CnW treatment, larger gaps got opened after mowing, where sufficient irradiance for germination was available for a longer period than in the relatively dense and homogeneous sward of CW mesocosms. This effect of waterlogging on gap formation after mowing was not observed in the non-canopy treatment where light was more effectively utilised by species of various growth forms. Interestingly, the inhibiting effect of waterlogging on total biomass production was much smaller for C treatments, which may reflect the adaptations of grasses to anaeroby, e.g. through the production of aerenchyma or shallow rooting.

The dominant role of interactions between canopy and other factors in controlling community assembly was also found by Xiong *et al.* (2003) in wet meadows but they did not include a typical stress factor, such as waterlogging in our experiment. Our results clearly show that the simultaneous response of species to waterlogging and competition results in a trade-off, leading to a strong synergic filtering effect when both types of stress occur simultaneously. Thus, our results indicate a hierarchical model, with canopy effects acting as a stronger filter than waterlogging, this in contrast to the results from Lenssen *et al.* (2003).

4.2. Functional mechanisms during early stages of community assembly

We found a close correlation (co-inertia) between species assembly patterns and traits related to germination and seedling establishment. These two stages are considered as major bottle necks for species recruitment (Grubb 1977, Shipley *et al.* 1989) and may largely determine patterns of biological diversity (Grime 1979, Henry *et al.* 2004). Moreover, because of their small stature, seedlings can be subject to a totally different light regime and soil resource availability than adult plants, even in the same site. Both wetness and light affected species germination in our experiment, but only few species were directly eliminated at this stage (1 under oxic and 3 under dark conditions).

It is clear that the main selection occurred at the next phase, i.e. establishment. None of the species could pass this stage if they germinated under grass canopy, unless mowing created a “light window” which allowed for a limited establishment of on average 10 species. This drastically asymmetric competition favoured species capable of withstanding shading. Successful species had a high biomass allocation to leaves, combined with a small individual size and a relatively low actual growth rate. These traits are typical of a stress-tolerant strategy, allowing plants to minimise resource requirements and survive in suboptimal conditions. Species that established under the canopy treatment had relatively small seeds. This is in contrast to expectations that large seeds with a large nutrient stock are advantageous in dense canopies, allowing seedlings to reach layers with higher light availability (Grime 1979). Among our species, there was no correlation between seed weight (SW) and ability for dark germination. A significant positive correlation between seed weight and early seedling height was indeed found but apparently even the largest seedlings could not reach layers with sufficient light.

When germinating on bare ground, seedlings of different species compete with each other for light, but the intensity of this competition is much higher in the non-waterlogged treatment. Traits responsible for rapid establishment and out-competing neighbours appear more important here than those responsible for shade tolerance (Keddy *et al.* 1994, Stockey & Hunt 1994). A combination of fast and massive germination with large-sized seedlings are prerequisites for success under dry conditions without imposed light stress, allowing *Plantago*, *Achillea*, and *Daucus* to dominate. Stockey & Hunt (1994) also found the germination rate in the first four days to be the best predictor of establishment success, whereas Körner *et al.* (2008) showed that small timing differences in species establishment may have large effects on later community composition. One exception among our species is *Phalaris*, which, despite a very limited germination, established well in both dry and wet mesocosms. Probably the unusual combination of high RGR and relatively large seedling size, together with the great ability for vegetative spread of established plants, may help to win the competition with small-seeded colonisers.

An unstressed environment during seedling germination and early establishment allowed for high dominance, which explains the significant reduction in functional richness when starting from aerobic conditions without canopy (nCnW). A computation of functional divergence on the basis of species presence-absence indeed resulted in much higher values for the nCnW treatments (not shown here). The dominance of a few competitive species prevented the development of functionally more divergent communities (i.e. enriched in specialized species).

In the presence of grass canopy much variation in functional divergence was observed. We believe that establishment under canopy leads to communities of individuals from randomly chosen species that establish by chance within a small canopy gap. This would also explain the high functional evenness in the canopy treatments where severe light stress acts as a strong filter allowing passage of only a few species, resulting in a limited functional width. At the same time the development of dominant species is also slowed down under such conditions. Communities under non-canopy conditions show significantly lower functional overlap, and contain several dominants.

4.3. Conclusions

Our results indicate that a certain level of environmental stress during community assembly may enhance functional richness by lowering competitive interactions between establishing plants. When operating together, waterlogging and competition have a strong synergic negative effect by reducing recruitment and community diversity, because of trade-offs between simultaneous responses to different types of stress.

The elimination of typical wetland specialists from dry mesocosms was clearly an effect of their poorer and/or slower germination and lower competitive abilities in comparison to non-wetland plants, leading to their disappearance in this low-stress environment. We suggest, therefore, that a high botanical diversity in wetlands may be largely the result of species recruitment in canopy gaps on waterlogged soils. A regular re-development of such gaps is normal in riverine wetlands that are subject to regular flooding. The colonisation of such gaps leads to a high functional richness only in combination with waterlogging, which impedes the growth of dominant species. Under eutrophic conditions an existing canopy may enhance diversity in the aerated soils through lowering the growth rate of the first colonisers.

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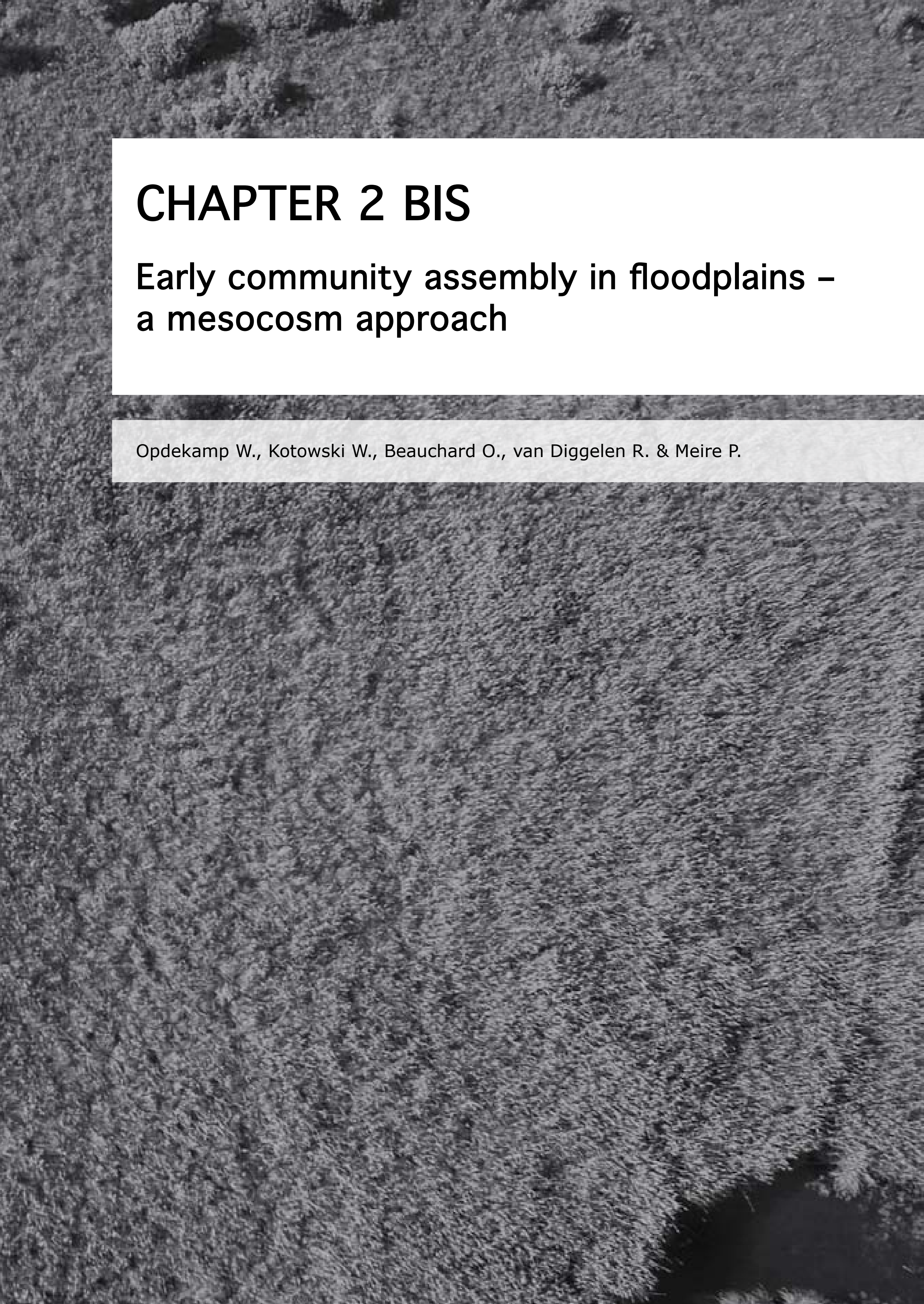
↓ **Appendix A.** Values of measured functional traits per species (for abbreviations and units, see Table 2)

→ **Appendix B.** Dry weight (g) of all species recorded in mesocosms in September 2006; n = 10.

Species	SW	GP	DGR	WGR	T50	AGR	RGR	H7	LWR7	LWR	LA7	LAR	SLA
Achillea millefolium	0.173	92.8	0.61	0.98	3	0.09	0.24	12	0.74	0.80	0.81	2.49	3.14
Alopecurus pratensis	0.700	72.0	0.95	0.80	4	0.03	0.18	97	0.51	0.67	1.54	4.72	10.43
Angelica sylvestris	2.456	2.0	0.60	0.34	-	-	-	-	-	-	-	-	-
Carex ovalis	0.492	82.0	0.01	1.50	5	0.01	0.21	30	0.92	0.88	0.34	4.82	5.76
Carex acuta	0.901	0.2	0.00	-	-	-	-	-	-	-	-	-	-
Centaurea jacea	2.327	43.2	0.86	1.32	3	0.13	0.18	20	0.74	0.75	2.81	2.45	3.33
Cirsium oleraceum	1.902	21.4	0.72	0.55	7	0.08	0.19	18	0.88	0.88	1.51	2.52	3.01
Cirsium palustre	1.112	50.0	0.32	0.26	6	0.04	0.18	7	0.80	0.86	0.78	2.72	3.28
Daucus carota	0.873	92.4	0.85	1.08	3	0.05	0.24	36	0.66	0.70	1.02	4.61	6.80
Epilobium hirsutum	0.106	80.0	0.42	1.20	3	0.02	0.20	11	0.87	0.88	0.58	5.15	5.99
Eupatorium cannabinum	0.220	51.4	0.25	5.07	9	0.05	0.30	11	0.87	0.86	0.48	5.22	6.11
Filipendula ulmaria	0.598	14.0	0.47	0.86	17	0.00	0.18	11	0.80	0.82	0.36	8.18	10.93
Galium verum	0.538	80.0	0.98	1.07	9	0.03	0.25	15	0.75	0.81	0.62	4.92	6.32
Geum rivale	1.214	46.8	0.25	0.89	9	0.02	0.21	12	0.77	0.78	0.72	4.47	5.85
Heracleum sphondylium	7.352	0.0	0.00	12.50	-	-	-	-	-	-	-	-	-
Leontodon autumnalis	0.802	65.6	1.05	1.16	4	0.15	0.24	28	0.76	0.77	1.94	3.14	4.13
Leucanthemum vulgare	0.427	26.8	0.68	1.07	5	0.07	0.22	17	0.81	0.84	0.93	2.57	3.09
Lolium perenne	0.900	95.2	1.00	0.86	3	0.08	0.22	113	0.69	0.74	1.80	3.60	6.37
Lychnis flos-cuculi	0.158	29.4	0.82	1.06	5	0.03	0.29	9	0.78	0.89	0.38	5.20	5.97
Lycopus europaeus	0.268	43.4	0.05	1.95	5	0.05	0.30	9	0.89	0.78	0.17	3.63	4.74
Lythrum salicaria	0.056	62.4	0.02	4.72	6	0.02	0.29	4	0.77	0.74	0.10	4.19	5.83
Myosotis palustris	0.280	26.0	1.05	1.38	7	0.03	0.21	8	0.79	0.81	0.69	4.27	5.55
Peucedanum palustre	1.350	1.6	0.00	1.68	-	-	-	-	-	-	-	-	-
Phalaris arundinacea	1.022	7.0	1.26	2.00	5	0.09	0.28	66	0.81	0.80	1.18	4.39	5.55
Plantago lanceolata	2.627	93.6	0.96	1.00	3	0.25	0.23	49	0.83	0.85	0.79	1.39	1.65
Poa pratensis	0.300	65.6	1.15	1.17	5	0.01	0.24	46	0.88	0.79	0.52	7.39	9.02
Prunella vulgaris	0.864	77.6	0.66	0.77	4	0.07	0.24	8	0.70	0.72	0.80	3.31	4.58
Ranunculus acris	1.789	41.6	0.36	0.81	13	0.03	0.24	11	0.77	0.73	0.59	3.76	5.15
Rumex acetosa	0.808	26.4	1.02	0.65	8	0.02	0.19	15	0.89	0.90	0.82	3.67	4.15
Senecio aquaticus	0.243	72.4	0.91	0.90	3	0.12	0.28	16	0.85	0.81	0.94	3.17	3.95
Typha latifolia	0.045	1.6	0.00	301.00	-	-	-	-	-	-	-	-	-
Urtica dioica	0.161	95.2	0.61	1.39	6	0.06	0.33	13	0.79	0.82	0.49	6.65	11.15
Valeriana officinalis	0.725	13.6	0.91	0.75	10	0.04	0.15	18	0.85	0.81	2.11	3.46	4.41
Veronica longifolia	0.072	86.6	0.91	0.71	4	0.02	0.26	7	0.84	0.93	0.31	6.47	7.31

	CWM		**CWnM**		**CnWM**		**CnWnM**		**nCWM**		**nCWnM**		**nCnWM**		**nCnWnM**	
	Mean	S.E.	**Mean**	S.E.	**Mean**	S.E.	**Mean**	S.E.	**Mean**	S.E.	**Mean**	S.E.	**Mean**	S.E.	**Mean**	S.E.
Dry weight whole sample	**582.287**	40.017	**1203.913**	35.664	**692.352**	39.267	**1751.940**	134.480	**626.447**	55.395	**1508.591**	88.670	**1344.776**	86.321	**2684.449**	181.091
Grass	**582.108**	40.017	**1203.913**	35.664	**689.350**	39.267	**1751.940**	134.480	**196.005**	24.650	**615.765**	21.568	**14.948**	4.056	**100.795**	25.116
Achillea millefolium	**0.000**	0.000	**0.000**	0.000	**0.001**	0.001	**0.000**	0.000	**2.266**	0.673	**7.892**	1.556	**20.008**	4.250	**285.664**	42.373
Angelica sylvestris	**0.004**	0.002	**0.000**	0.000	**0.007**	0.003	**0.000**	0.000	**0.005**	0.003	**0.006**	0.003	**0.004**	0.003	**0.000**	0.000
Centaurea jacea	**0.044**	0.020	**0.000**	0.000	**1.169**	0.619	**0.000**	0.000	**8.342**	1.363	**11.464**	1.830	**10.456**	3.208	**35.493**	11.927
Cirsium oleraceum	**0.017**	0.010	**0.000**	0.000	**0.198**	0.060	**0.000**	0.000	**16.111**	4.086	**22.187**	4.341	**0.740**	0.538	**5.655**	2.111
Cirsium palustre	**0.004**	0.002	**0.000**	0.000	**0.028**	0.011	**0.000**	0.000	**0.674**	0.208	**2.823**	0.563	**0.012**	0.012	**0.036**	0.036
Daucus carota	**0.023**	0.010	**0.000**	0.000	**0.990**	0.390	**0.000**	0.000	**10.768**	2.292	**16.664**	1.913	**95.672**	21.866	**243.649**	52.126
Epilobium hirsutum	**0.000**	0.000	**0.000**	0.000	**0.001**	0.001	**0.000**	0.000	**0.037**	0.024	**32.488**	7.637	**0.000**	0.000	**8.662**	6.696
Eupatorium cannabinum	**0.000**	0.000	**0.000**	0.000	**0.003**	0.002	**0.000**	0.000	**0.007**	0.003	**0.013**	0.006	**0.000**	0.000	**0.025**	0.024
Galium verum	**0.000**	0.000	**0.000**	0.000	**0.001**	0.001	**0.000**	0.000	**0.049**	0.023	**0.148**	0.040	**0.070**	0.029	**2.771**	0.483
Geum rivale	**0.002**	0.001	**0.000**	0.000	**0.001**	0.001	**0.000**	0.000	**0.002**	0.002	**0.005**	0.003	**0.001**	0.001	**0.000**	0.000
Leontodon autumnalis	**0.000**	0.000	**0.000**	0.000	**0.013**	0.006	**0.000**	0.000	**12.441**	2.533	**10.924**	1.525	**0.225**	0.048	**6.770**	2.046
Leucanthemum vulgare	**0.000**	0.000	**0.000**	0.000	**0.003**	0.002	**0.000**	0.000	**0.051**	0.017	**0.027**	0.017	**0.000**	0.000	**0.000**	0.000
Lychnis flos-cuculi	**0.000**	0.000	**0.000**	0.000	**0.000**	0.000	**0.000**	0.000	**0.002**	0.002	**0.000**	0.000	**0.000**	0.000	**0.000**	0.000
Lycopus europaeus	**0.018**	0.010	**0.000**	0.000	**0.127**	0.069	**0.000**	0.000	**0.688**	0.207	**11.738**	3.088	**0.048**	0.048	**0.238**	0.238
Lythrum salicaria	**0.028**	0.020	**0.000**	0.000	**0.042**	0.021	**0.000**	0.000	**3.048**	1.064	**99.512**	18.122	**0.143**	0.143	**0.000**	0.000
Myosotis palustris	**0.001**	0.001	**0.000**	0.000	**0.003**	0.002	**0.000**	0.000	**0.062**	0.031	**0.976**	0.128	**0.000**	0.000	**0.095**	0.073
Phalaris arundinacea	**0.000**	0.000	**0.000**	0.000	**0.000**	0.000	**0.000**	0.000	**157.226**	33.547	**262.883**	46.756	**217.666**	42.783	**404.817**	76.899
Plantago lanceolata	**0.013**	0.010	**0.000**	0.000	**0.220**	0.131	**0.000**	0.000	**171.719**	32.645	**168.264**	33.585	**887.029**	68.318	**1167.943**	51.714
Prunella vulgaris	**0.015**	0.010	**0.000**	0.000	**0.048**	0.022	**0.000**	0.000	**0.005**	0.003	**0.080**	0.050	**0.000**	0.000	**0.000**	0.000
Ranunculus acris	**0.008**	0.003	**0.000**	0.000	**0.005**	0.002	**0.000**	0.000	**0.663**	0.259	**0.858**	0.257	**0.000**	0.000	**0.019**	0.018
Rumex acetosa	**0.002**	0.002	**0.000**	0.000	**0.103**	0.071	**0.000**	0.000	**1.601**	0.698	**1.060**	0.265	**4.524**	1.347	**13.896**	8.733
Senecio aquaticus	**0.000**	0.000	**0.000**	0.000	**0.002**	0.001	**0.000**	0.000	**6.540**	1.593	**4.749**	2.068	**3.482**	3.469	**0.024**	0.024
Typha latifolia	**0.000**	0.000	**0.000**	0.000	**0.000**	0.000	**0.000**	0.000	**4.250**	3.928	**8.774**	3.555	**0.000**	0.000	**0.000**	0.000
Urtica dioica	**0.001**	0.001	**0.000**	0.000	**0.038**	0.022	**0.000**	0.000	**0.001**	0.001	**0.011**	0.007	**0.902**	0.896	**2.779**	2.485
Valeriana officinalis	**0.000**	0.000	**0.000**	0.000	**0.000**	0.000	**0.000**	0.000	**0.002**	0.002	**0.006**	0.003	**0.000**	0.000	**0.000**	0.000



CHAPTER 2 BIS

Early community assembly in floodplains – a mesocosm approach

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1. INTRODUCTION

The ability to predict the composition of communities from environmental factors is a central goal of community ecology (Weiher & Keddy 1995a). Vegetation ecologists agree on the central role of competition in community assembly under physically optimal conditions and high resource availability, e.g. in mesic grasslands or old fields (e.g. Grime 1979, Walker & Chapin 1986, Tilman 1988), and the dominance of habitat filtering in more extreme habitats, such as deserts or salt marshes (Grime 1979). However, much more uncertainty exists regarding the relative importance of competition and habitat filtering when stress and resource gradients coincide with each other. This question is of particular interest in dynamic ecosystems subjected to frequent disturbance and high rates of seed immigration, where community re-assembly is a frequent process structuring vegetation patterns (Myers & Harms 2009).

In Chapter 2, we presented the results of a mesocosm experiment on early community assembly in river floodplains. In riparian landscapes, species distribution has been shown to be jointly controlled by water level and competition (Weiher & Keddy 1995b, Lenssen *et al.* 2003, Kotowski & van Diggelen 2004, Kotowski *et al.* 2006). In the initial phase of community assembly, canopy presence was a much stronger filtering factor, almost totally disabling establishment (Chapter 2). This effect could only be reduced slightly by mowing, which opened the canopy for a short time period. On the other hand, waterlogging had a lesser effect on community composition and did not exclude any species from the community when acting as a sole stress factor. It even allowed for the highest species richness in the absence of a closed canopy at the moment of seed addition. Yet, waterlogging interacted with grass canopy presence. When both stress factors acted together, species richness was further reduced. This dominant role of interactions between canopy and other factors in controlling community assembly was also found by (Xiong *et al.* 2003)in wet meadows but they did not include a typical stress factor, such as waterlogging. The results presented in Chapter 2 thus clearly showed how the simultaneous response of species to waterlogging and competition resulted in a trade-off, leading to a strong synergic filtering effect when both types of stress occurred simultaneously. Hence, this would indicate a hierarchical model, with canopy effects acting as a stronger filter than waterlogging, contrasting with the results from

(Lenssen *et al.* 2003) and with existing hierarchical model of environmental filters, where habitat filtering is considered to be dominant over competition (Zobel *et al.* 1998).

According to Chapter 2, community assembly in river floodplains seems to be mainly driven by the competition filter. In this appendix, we tested whether the relative impact of biotic and abiotic filters, operating on community composition in a river floodplain, changed over time.

2. METHODS

2.1 SELECTED SPECIES

We selected 34 species that occur frequently in floodplains of temperate Europe and represent the whole moisture gradient from mesic meadows to reed beds and swamps (Chapter 2, Table 1). We deliberately selected species with different seed size within each moisture category. Seeds were obtained from commercial seed suppliers (Ecoflora, Halle, Belgium; De Bolderik, Wervershoof, the Netherlands; De Morgenster-zaden, Bussum, the Netherlands) as ‘ready-to-use’.

2.2 SCREENING FOR FUNCTIONAL TRAITS

The screening of the functional traits is described in Chapter 2.

2.3 MESOCOSM EXPERIMENT

We established a mesocosm experiment, followed during three consecutive years (2006 — 2008, in open air to study the impact of waterlogging, canopy-shading and a temporal alleviation of light stress through mowing on the first phases of establishment of herbaceous plant communities. The mesocosms consisted of PVC containers (111 x 91 x 61 cm height), filled with a drainage substrate for 25 cm (Argex clay aggregate, Argex NV, Zwijndrecht, Belgium) separated by geo-textile from a 25 cm cover of a soil mixture (3:1 volumetric parts of fen peat and alluvial clay). To ensure eutrophic conditions throughout the experiment, an initial amount of 168g N, 192g P, 216g K and 24g Mg (PG-mix Floranid, COMPO Benelux nv, Deinze, Belgium) per m^3 soil, and a slow release fertilizer (6 months release time; Osmocote, SCOTTS Professional, Sint-Niklaas, Belgium) containing 300g N, 180g P and 180g K per m^3 soil were added. These values are at the upper limit of annual nutrient input in European floodplains (Pinay *et al.* 1995, Olde Venterink *et al.* 2006). Eighty mesocosms were positioned in a block of eight rows. Water was applied through sprinklers installed 30 cm above each container once (spring, autumn) or twice (summer) a day.

We applied the following treatments in a full-factorial design with 10 replicates for each treatment combination (given abbreviations are used later in the text):

C/nC – germination under canopy of *Poa pratensis*, *Lolium perenne* and *Alopecurus pratensis* vs. germination on bare ground; grasses were pre-grown for 6 weeks and cut at 10 cm height at the sowing date (the species mixture ensured that canopy persisted under both dry and wet conditions);

W/nW – waterlogged treatment with water level maintained 5 cm below soil surface vs. non-waterlogged (aerated) treatment with water percolation through soil profile and air penetration from below;

M/nM – mowing of vegetation at 2cm height at the end of June 2006, 2007 and 2008 vs. no mowing.

Thirty-four species were sown into the mesocosms on May 15th 2006 with a density of c.1000 seeds species^{-1} m^{-2}. *Poa*, *Alopecurus* and *Lolium* were used as matrix vegetation in the C treatment but were also part of the species pool introduced to all containers. The number of seeds was determined by weight, after establishing the weight of 100 seeds with an accuracy of 0.001 g. The total number of seeds, 37000 m-2, was similar to the average seed production on wet meadows (Klimkowska *et al.* 2009).

During the experiment, an AccuPAR LP-80 Ceptometer (Decagon Devices Inc., Pullman, USA) was used to measure light attenuation in all mesocosms at 0 and 10 cm above ground surface.

During late August — early September 2006, 2007 and 2008, above-ground biomass of all mesocosms was harvested and sorted to species level (except for Poa, Lolium and Alopecurus, which were grouped together). Throughout the experiment, care was taken to remove incidental individuals of non-introduced species, however if some of them remained in the communities, they were separated during harvesting. Dry mass was measured after 72 hours of drying at 70 °C.

2.4 DATA ANALYSIS

Vegetation indices

Treatment effects on species richness (excluding the three matrix grass-species) were analysed with a generalised linear model with Poisson distribution (zero-inflated for the analysis of the first year results). Treatment effects on aboveground biomass of the tested species was tested using a linear model after log (x + 1) transformation. Both analyses were performed using R (R Development Core Team 2011).

Species community pattern and relationship with biological traits

The biomass of all tested species was arranged in an 80 containers × 28 species table, separately for each year, to explore species community patterns through a centred PCA, after log (x + 1) transformation.

Subsequently, permutational multivariate analysis of variance (PERMANOVA: Anderson 2001) was used to analyse the effect of the experimental factors on the floristic community structure. This method is more appropriate than traditional methods such as MANOVA to analyze changes in composition as it can be based on any ecologically relevant distance metric and not solely on Euclidean distances (McArdle & Anderson 2001). Furthermore, *p*-values are derived from permutations of the raw data or the residuals, whereby the data do not have to conform to a multivariate normal distribution. Given that the distributions of abundances of individual species are often highly skewed or aggregated (e.g. Gaston & McArdle 1994), this broadens the applicability of PERMANOVA compared to MANOVA. We used the Euclidean distance metric and floristic data were log (x+1) transformed prior to analysis.

The relationship between the species × traits table (trait-columns as species ability scores) and the transposed species × treatments table (treatment-columns as species responses) was tested using a Co-Inertia Analysis (CIA: Dray *et al.* 2003). CIA constructs axes encompassing only the common ecological information between both patterns, and the statistical significance of the link was tested based on 9999 random permutations of the rows of the two tables.

Computations and associated graphical representations were implemented using the vegan (Oksanen *et al.* 2010) and ade4 packages (Dray & Dufour 2007) available in R freeware (R Development Core Team 2011).

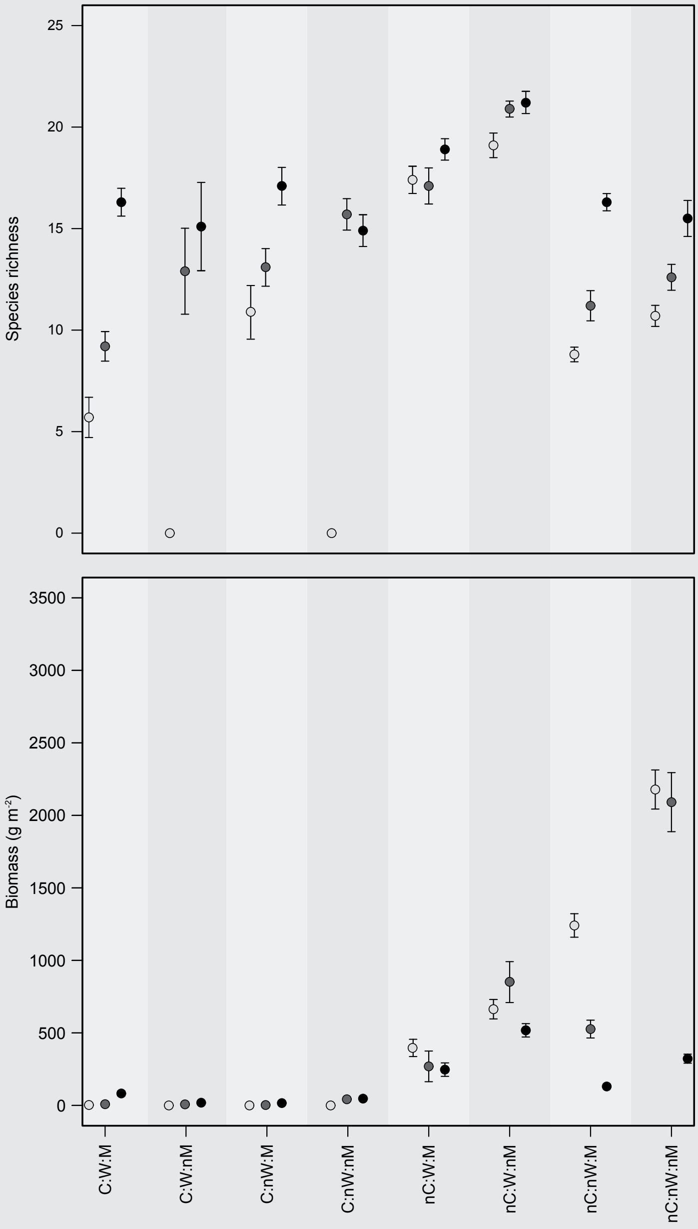
3. RESULTS AND DISCUSSION

3.1 Effects on species establishment

In the first year of the experiment (2006), seedling establishment of all species was drastically suppressed in C treatments, decreasing species number as well as biomass (Fig. 1, Table 1, Table 2). Although germination was observed here, all seedlings died within a few weeks. This effect was slightly lowered by mowing, resulting in limited establishment of few species in CM (cf. interaction C × M, Table 3). In terms of biomass production this establishment was still negligible compared to the nC treatment (Fig. 1, Table 2), where multi-species plant communities developed (Fig. 1). Waterlogging interacted with canopy controlling species richness. Under canopy, where tested species only established after mowing, waterlogging decreased the number of species from 12 (CnWM) to 6 (CWM). The opposite situation was found in the absence of canopy, where nCW mesocosms contained twice as many species as the nCnW. Growth of the established species was largely reduced by waterlogging (Fig. 1, Table 2); this effect was much stronger in C (after mowing) (waterlogging decreased biomass of tested species from 3.0 g m^{-2} to 0.18 g m^{-2}, i.e. by 94%) than in nC treatment (decrease by 66%).

During the subsequent years of the experiment (2007, 2008), a general increase in species richness could be observed until most treatments supported on average 15 species. Yet, species richness in waterlogged mesocosms without initial grass canopy cover remained highest (Fig. 1, Table 1).

The first year results indicated a hierarchical model (Chapter 2), with canopy effects acting as a stronger filter than waterlogging, contrary to the results of (Lenssen *et al.* 2003). Yet, the relative importance of waterlogging and initial vegetation cover as drivers of species community composition changed during the consecutive years of the mesocosm experiment. This is concordant with the existing hierarchical model of environmental filters, where habitat filtering is considered to be dominant over competition (Zobel *et al.* 1998, Lenssen *et al.* 2003).



↑ **Fig 1.** Species richness and total biomass in response to different treatments during the three consecutive years of the experiment (C: canopy, W: waterlogging, M: summer-mowing, n: no, 2006: ○, 2007: ●, 2008: ●, shown are means ± SE).

↓ **Table 3.** Z or t-values and significance for treatment effects on species richness; significance: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Significant effects are indicated in bold. See also Fig.1

2006	Treatment	Z
	C	0.932^{ns}
	W	**3.315^{***}**
	M	-0.004^{ns}
	C×W	**6.258^{***}**
	C×M	0.004^{ns}
	W×M	0.000^{ns}
	C×W×M	0.000^{ns}

2007	Treatment	t
	C	**-2.409^{*}**
	W	**-3.277^{**}**
	M	**-3.785^{***}**
	C×W	**6.659^{***}**
	C×M	-0.010^{ns}
	W×M	0.000^{ns}
	C×W×M	0.000^{ns}

2008	Treatment	t
	C	-0.098^{ns}
	W	-0.296^{ns}
	M	-1.508^{ns}
	C×W	**2.849^{***}**
	C×M	0.676^{ns}
	W×M	0.472^{ns}
	C×W×M	0.303^{ns}

↓ **Table 4.** F-values and significance for treatment effects on aboveground biomass; df = degrees of freedom; significance: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. Significant effects are indicated in bold. See also Fig.1

2006	Treatment	*Df*	SSqs	MeanSqs	*F*
	C	**1**	**830.32**	**830.32**	**5998.75*****
	W	**1**	**15.08**	**15.08**	**108.95*****
	M	1	0.02	0.02	0.13^{ns}
	C×W	**1**	**2.75**	**2.75**	**19.85*****
	C×M	**1**	**1.36**	**1.36**	**9.85****
	W×M	**1**	**7.68**	**7.68**	**55.49*****
	C×W×M	**1**	**1.12**	**1.12**	**8.09****
	Residuals	72	9.97	0.14	

2007	Treatment	*Df*	SSqs	MeanSqs	*F*
	C	**1**	**420.41**	**420.41**	**817.91*****
	W	**1**	**35.56**	**35.56**	**69.19*****
	M	**1**	**30.10**	**30.10**	**58.56*****
	C×W	**1**	**2.61**	**2.61**	**5.08***
	C×M	1	0.77	0.77	1.50^{ns}
	W×M	**1**	**2.29**	**2.29**	**4.46***
	C×W×M	**1**	**2.47**	**2.47**	**4.81***
	Residuals	72	37.01	0.51	

2008	Treatment	*Df*	SSqs	MeanSqs	*F*
	C	**1**	**101.06**	**101.06**	**242.22*****
	W	1	1.28	1.28	3.06^{ns}
	M	1	1.00	1.00	2.40^{ns}
	C×W	1	1.33	1.33	3.19^{ns}
	C×M	**1**	**7.73**	**7.73**	**18.52*****
	W×M	**1**	**9.61**	**9.61**	**23.04*****
	C×W×M	**1**	**8.12**	**8.12**	**19.47*****
	Residuals	72	30.04	0.42	

↓ **Table 5.** Treatment effects on the community composition during three consecutoive years using permutational multivariate ANOVA, with Euclidean distances and log+1 transformed data; Treatment abbreviations (C: canopy, W: waterlogging, M: summer-mowing); significance levels (ns: not significant, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$)

2006	Treatment	*Df*	SSqs	MeanSqs	*F*	partialR^2
	C	1	2018.9	2018.9	454.9	0.70***
	W	1	194.3	194.3	43.8	0.07***
	M	1	54.6	54.6	12.3	0.02***
	C×W	1	181.5	181.5	40.9	0.06***
	C×M	1	66.7	66.7	15.0	0.02***
	W×M	1	26.7	26.7	6.0	0.01**
	C×W×M	1	30.7	30.7	6.9	0.01**
	Residuals	72	319.5	4.4		0.11
	Total	79	2892.9			1.00

2007	Treatment	*Df*	SSqs	MeanSqs	*F*	partialR^2
	C	1	1305.2	1305.2	244.3	0.51***
	W	1	333.9	333.9	62.5	0.13***
	M	1	154.6	154.6	28.9	0.06***
	C×W	1	173.6	173.6	32.5	0.07***
	C×M	1	107.6	107.6	20.2	0.04***
	W×M	1	36.2	36.2	6.8	0.01**
	C×W×M	1	43.4	43.4	8.1	0.02***
	Residuals	72	384.6	5.3		0.15
	Total	79	2539.2			1.00

2008	Treatment	*Df*	SSqs	MeanSqs	*F*	partialR^2
	C	1	633.2	633.2	83.6	0.31***
	W	1	412.7	412.7	54.5	0.20***
	M	1	87.1	87.1	11.5	0.04***
	C×W	1	138.5	138.5	18.3	0.07***
	C×M	1	87.7	87.7	11.6	0.04***
	W×M	1	48.8	48.8	6.4	0.02***
	C×W×M	1	64.0	64.0	8.4	0.03***
	Residuals	72	545.7	7.6		0.27
	Total	79	2017.6			1.00

3.2 Species community pattern

In the first year, the first PCA axis opposes the C mesocosms to those with nC treatments (Fig. 2). The conjoined effect of the three controlled factors explained most of the observed pattern. The canopy effect was found to be highly significant, indicating extremely low establishment of species in C treatments, thus engendering a biomass gradient characterized by positive species covariances on the first axis. The second axis highlights a divergence between species covariances, corresponding to a waterlevel gradient, particularly in nC treatments. The effect of waterlogging clearly separated wetland species from non-wetland species (as indicated by Ellenberg index). The mowing effect alone was found to be low. Its influence is limited to the conjoined effect where establishment is slightly enhanced in C conditions and where biomass is reduced in nC conditions, particularly in W treatments. The major effect of grass canopy cover, compared to the other treatments is confirmed by the PERMANOVA-analysis (Table 3).

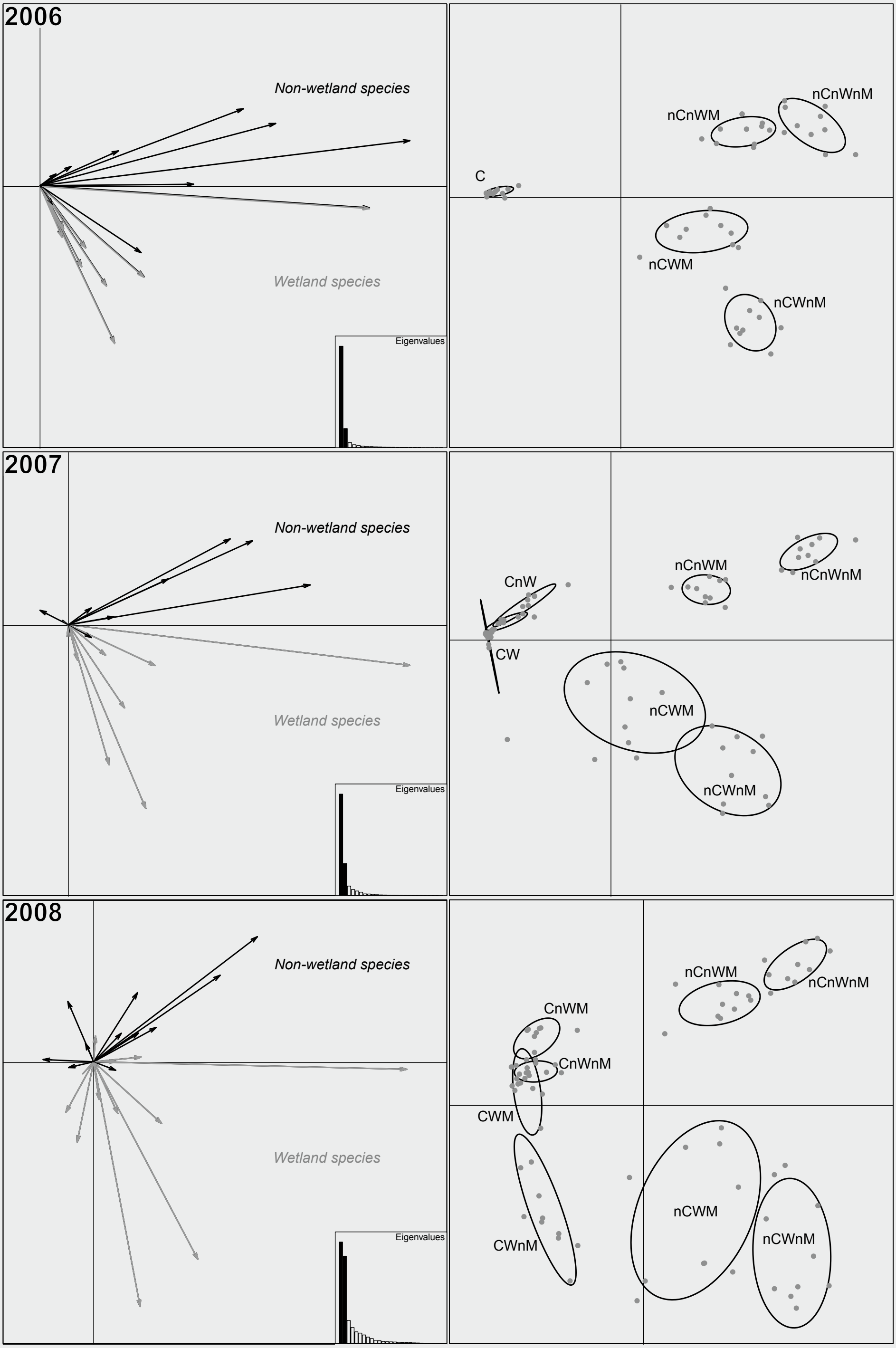
During the consecutive years, this pattern changed. In 2007 and 2008, the first axis of the PCA still opposes the C mesocosms to those with nC treatments. In contrast to the first year, more species established in the C mesocosms, confirmed by some negative species covariances with the first axis. Yet, waterlogging gained importance as an explanatory factor of plant community composition as shown by the increased eigenvalues of the second axis (Fig. 2). The increased relative importance of waterlogging compared to grass canopy presence as explanatory factor was also confirmed by the partial R^2 for both factors in the PERMANOVA-analysis (Table 3).

In the first year, the co-inertia analysis (Fig. 3) revealed a highly significant co-structure between biological traits and species responses ($p < 0.0001$). The first factorial plane encompassed respectively 68.1 and 98.0% of both inertia patterns. The first axis encompassed species' responses to canopy effect, characterized by high scores of seedling height (H7), actual growth rate (AGR), seed weight (SW), and mean leaf area of seedlings at 7th day (LA7) in nC conditions, opposed to slower germination (high T50) and high scores of both LAR at day 7 (LAR7) and increase in LAR between 7th and 22nd day (LAR), SLA and increase in LWR between 7th and 22 day (LWR) in C conditions. On the second CIA axis, W conditions were characterized by high LWR at day 7 (LWR7), good germination in wet mesocosms (WGR) and high RGR, opposed to high ability for dark germination (DGR) and high total germination percentage in light (GP) in nW conditions.

This pattern was preserved to a large extent during the second and third year (Fig. 3; 2007, $p = 0.0025$; 2008, $p = 0.0061$). The first factorial plane encompassed respectively 68.2 and 98.0% (2007) and 73.6 and 94.3% (2008) of the inertia patterns of biological traits and species responses. Yet, the distribution of pseudo-eigenvalues for the first and second axis differs strongly for third year as compared to the first and second year. In the third year, more inertia was encompassed along the second axis, indicating a stronger link between species responses and traits related to established in wet versus dry conditions as opposed to the first and second year.

Hence, species and trait patterns remained interrelated during the consecutive years of the mesocosm experiment. This further stresses the importance of the germination and establishment phase for determining diversity patterns (Grime 1979, Henry *et al.* 2004). The increased linkage between waterlogging and traits related to germination and establishment is supportive for a hierarchical model of environmental filters, where habitat filtering is considered to be dominant over competition (Zobel *et al.* 1998, Lenssen *et al.* 2003).

→ **Fig 2.** Species community patterns in different years (PCA on treatments × species table); Left panel: species projections, split according to Ellenberg moisture indicator into non-wetland and wetland species, respectively 4 to 6 (black) and 7 to 10 (grey), and eigenvalues diagram; Right panel: projections of the mesocosms, grouped per treatment (C: canopy, W: waterlogging, M: summer-mowing, n = no; 2006: F1 73.9%, F2 13.9%; 2007: F1 61.2%, F2 19.4%; 2008: F1 35.2%, F2 30.3%).


2006
Non-wetland species
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nCnWnM
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2008
Non-wetland species
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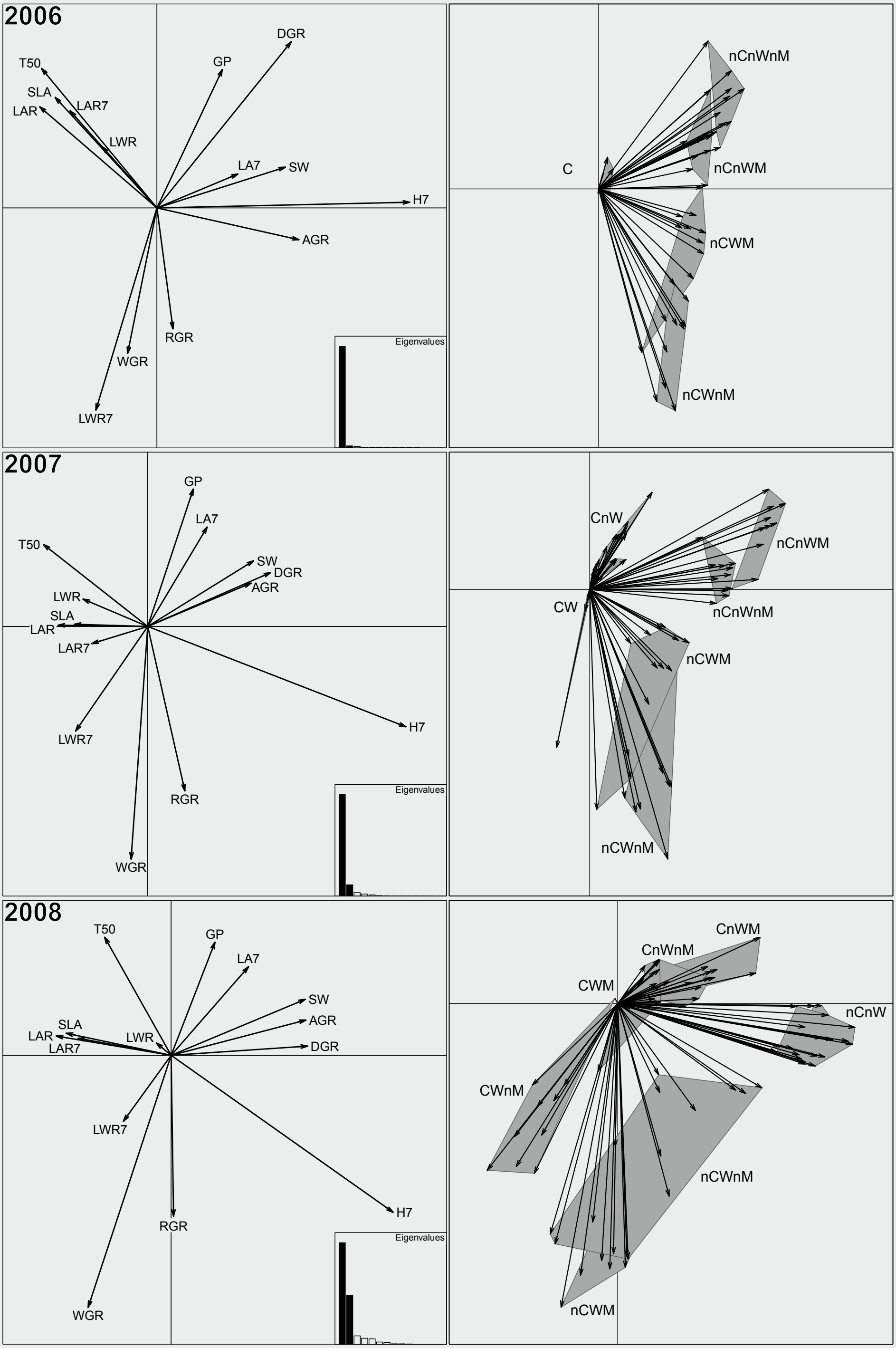
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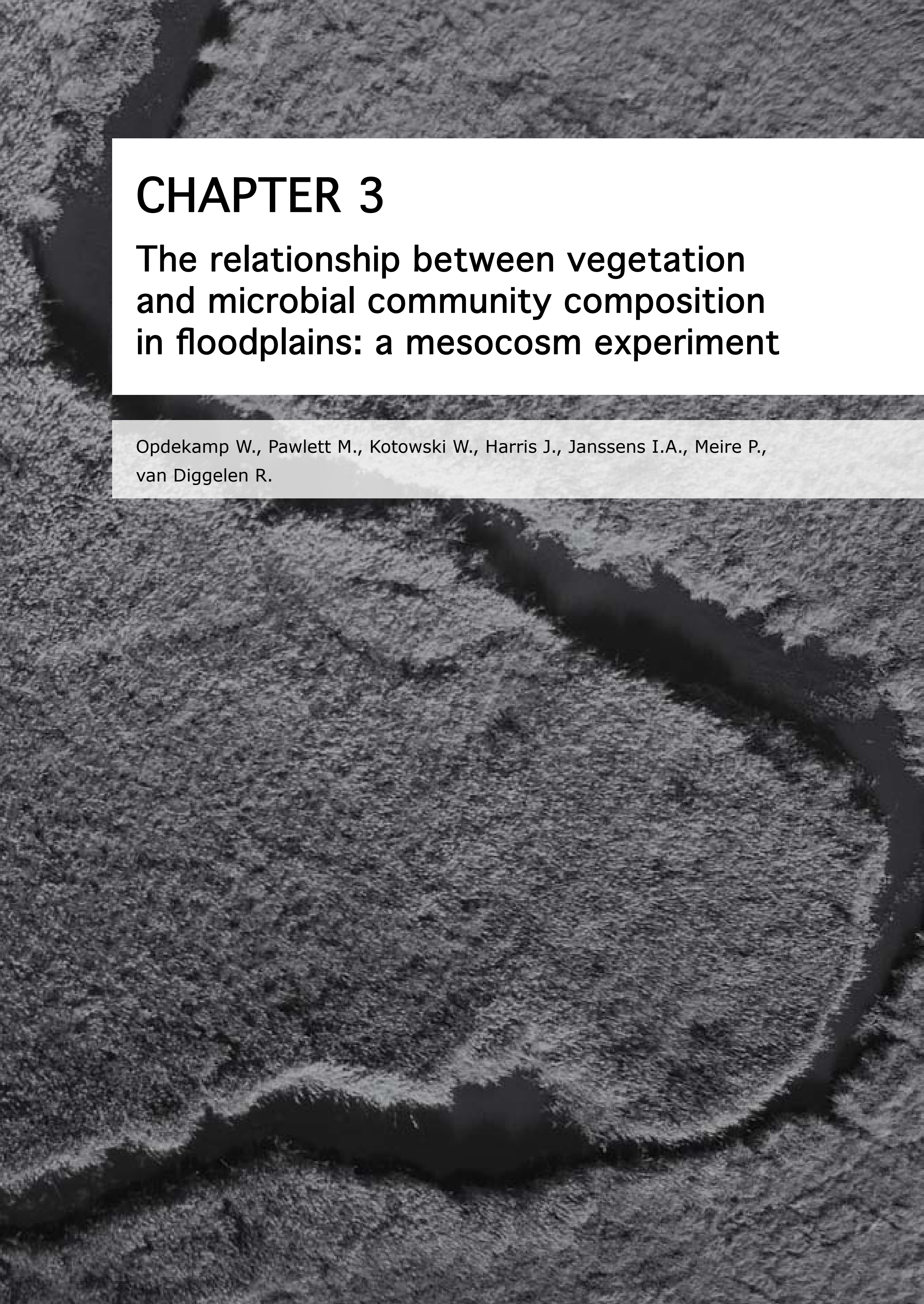
LWR
LAR7
LWR7
H7
RGR
WGR
Eigenvalues
CnWM
CnWnM
CWM
nCnW
CWnM
nCWnM
nCWM

← **Fig 3.** Biological responses to treatments in different years, using a co-inertia analysis between biological traits (Left panel) and tratments (Right panel). For trait abbreviations see Chapter 2, Table 2 (C: canopy, W: waterlogging, M: summer-mowing, n = no).



CHAPTER 3

The relationship between vegetation and microbial community composition in floodplains: a mesocosm experiment

Opdekamp W., Pawlett M., Kotowski W., Harris J., Janssens I.A., Meire P., van Diggelen R.

ABSTRACT

Assuming that the hydrological regime and competition for light are two major environmental sieves in riparian plant communities, we examined in the present study whether the same holds true for microbial communities and tried to explore the interactions between these factors operating at both communities. Furthermore, as vegetation and soil microbial community composition are intrinsically linked with both positive and negative feedbacks, we used co-correspondence analysis to predict the composition of one community based on the composition of the other.

To answer our questions, we carried out a mesocosm experiment that focused on three factors: i) the impact of waterlogging, ii) the effect of a dense vegetation cover at initial inoculation and iii) the effect of a temporal alleviation of light stress.

Our results clearly demonstrated that the experimental treatments affected the composition of both the microbial and plant communities. Of these experimental parameters, waterlogging had the largest effect. Interestingly, the effect of the grass canopy cover was more pronounced in the floristic than in the microbial community. Furthermore, at this initial stage of community development the floristic community seemed to have a stronger effect on the microbial community than vice versa. The outcomes of our study confirm the difficulties in predicting aboveground consequences of belowground interactions and vice versa.

1. INTRODUCTION

Vegetation and soil microbial community composition are intrinsically linked (Wardle *et al.* 2004) with both positive and negative feedbacks (Wardle 2002, Reynolds *et al.* 2003, Wardle *et al.* 2004, van der Heijden *et al.* 2008, Kardol & Wardle 2010). Vegetation composition may directly affect both the quality and quantity of organic matter available for soil microbial decomposition, thereby influencing microbial community composition (Zak *et al.* 2003). More in particular, fungal/bacterial ratios were observed to change in response to altered ratios of recalcitrant versus easily decomposable organic matter (Wardle *et al.* 2004), microbial biomass and activity change with differing composition of root exudates (Grayston *et al.* 1996), and microbial activity can be altered by plant-induced changes in soil moisture and pH (Paul & Clark 1996). Conversely, soil micro-organisms may affect vegetation composition through microbial-driven modifications of resource competition between plant species and through species-specific responses of plants to individual microbial species (microbial pathogens and positive root symbionts are often species- or host-specific) (van der Heijden *et al.* 2008, Bever *et al.* 2010). Consequently, the microbial community composition can have strong direct effects on vegetation composition and vice versa.

Factors affecting microbial community composition in grassland, heathland and woodland soils have been studied repeatedly (e.g. Stephan *et al.* 2000, Mitchell *et al.* 2010, Nielsen *et al.* 2010). In wetland soils, hydrological conditions appeared to be a dominant factor, affecting soil microbial community structure in a complex way, with different components of the microbial community responding differently (Sundh *et al.* 1997, Bossio & Scow 1998, Mentzer *et al.* 2006, Rinklebe & Langer 2006, Jaatinen *et al.* 2007, Unger *et al.* 2009). Soil fungal biomass for instance reduced strongly upon flooding (Unger *et al.* 2009). Hydrological gradients are known to be a major driver of plant distribution in wetland systems (Grootjans 1980, Pałczyński 1984). Interestingly, few studies have investigated soil moisture content as a common driving factor for both the soil microbial and plant community composition (Balasooriya *et al.* 2008, Eskelinen *et al.* 2009, Yu & Ehrenfeld 2010) or focused on plant-microbial interactions in freshwater wetland soils (Hartman *et al.* 2008). In addition to hydrology, other abiotic factors in wetlands that have a major impact on both vegetation and soil microbial community composition in wetlands include pH (Olde Venterink *et al.* 2001, Hartman *et al.* 2008, Eskelinen *et al.* 2009) and nutrient availability (Bridgham *et al.* 1996, Jonasson *et al.* 1999). Micro-organisms show distinct preferences soil organic matter composition. A bacteria-dominated soil microbial community is often related to low C:N and high soluble N:phenolics ratios in the soil organic matter whereas a high proportion of fungi within the microbial community is linked to high C:N and low soluble N:phenolics ratios (Wardle *et al.* 2004, Eskelinen *et al.* 2009). Enhanced nutrient availability also leads to increased above-ground productivity and greater competition for light between plants (Aerts 1999, Kotowski & van Diggelen 2004). Hence, mowing leads to a temporal relief of above-ground competition, enabling enhanced seedling recruitment (Kotowski *et al.* 2010) and potentially affects microbial community structure.

Interactions among factors that are likely to alter plant and microbial species composition in wetland systems are complex. Long-term hydrological changes will invariably affect soil abiotic factors such as pH and nutrient status (Lamers *et al.* 2006, Olde Venterink *et al.* 2009). Therefore we carried out an experiment that focused on three factors: i) the impact of waterlogging, ii) the effect of a dense vegetation cover at initial inoculation and iii) the effect of a temporal alleviation of light stress. The objectives of this study were hence to determine a) whether the composition of microbial and plant communities is affected by the experimental treatments, b) whether plant and microbial communities react different on changes in these treatments, and c) to what degree plant and microbial communities affect each other.

2. METHODS

2.1 Experimental design

An outdoor mesocosm experiment was constructed with 80 PVC-containers (W × L × H: 111 × 91 × 61 cm), comprising of a 25 cm drainage layer (Argex clay aggregate, Argex NV, Zwijndrecht, Belgium) covered with a geo-textile boundary and then 25 cm soil. The soil consisted of a homogenised mixture of fen peat and alluvial clay (3:1 v/v).

Experimental treatments were i) Waterlogging (with and without waterlogging), ii) Canopy presence (with and without canopy) at initial inoculation, iii) Mowing (with and without summer mowing) for a full-factorial design (2 x 2 x 2) with 10 replicates.

We selected 34 plant species that occur frequently in floodplains of temperate Europe and represent the entire moisture gradient from mesic meadows to reed beds and swamps (see Appendix A for species list). Seeds were sown in all mesocosms at a rate of approximately 1000 seeds species^{-1} m^{-2} on May 15th 2006. The seeding rate was similar to seed production on wet meadows (Klimkowska *et al.* 2009). The canopy (C/nC) consisted of *Poa pratensis*, *Lolium perenne* and *Alopecurus pratensis*. These grasses were used as matrix vegetation in the canopy treatment but were also part of the species pool introduced to all containers. They were pre-grown for 6 weeks and cut at 10 cm height when the other seeds were added. This grass species mixture ensured the persistence of a grass canopy in both dry and wet conditions. In other mesocosms, soil was kept bare until the sowing date. Waterlogging (W/nW) was maintained at 5 cm below soil surface. Non-waterlogged soils were allowed to drain freely. Mowing (M/nM) involved annual mowing of vegetation to 2 cm in June–July. A more detailed description of the experimental set-up can be found in Kotowski *et al.* (2010).

Above-ground plant biomass was harvested in August of 2006, 2007 and 2008. The harvest was sorted to species level with the exception of *Poa, Lolium and Alopecurus* which were grouped together. Dry mass was determined after 72 h of drying at 70°C. Only the 2008 data are used for analysis in the current study.

2.2 Soil Sampling

In 2008 three soil cores (□ 3 cm x 10 cm depth) were procured from each mesocosm. Soil was stored at 4°C and processed within 48 h. The three cores were homogenised, roots removed, and then divided in two sub-samples to determine extractable nutrients and the composition of the soil microbial community.

2.3 Nutrient analysis

Soil extractable nitrogen (NO_2^-, NO_3^- & NH_4^+) was determined after extraction of 10g wet soil with 25mL of 1M KCl (Houba *et al.* 1989). PO_4^{3-}, Na, K, Ca, and Mg were determined following extraction of 5 g soil (fresh weight) with 25 mL of a 0.5 M ammonium acetate-EDTA (pH 4.65) (Cottenie *et al.* 1989). NO_x^-, NH_4^+ and PO_3^{4-} were determined colorimetrically using a Skalar auto-analyser (Skalar, Breda, The Netherlands). Na, K, Ca, and Mg concentrations were determined using a Thermo Iris ICP (Thermo Fisher Scientific, Waltham, USA). pH was determined using a soil:water ratio of 1:2.5 (w:v) (Houba *et al.* 1989).

2.4 Microbial community composition

Phospholipid fatty acid (PLFA) analysis was used to observe the phenotypic profiles of the soil microbial community and was performed according to Frostegård (Frostegård *et al.* 1993). Lipids were extracted from approximately 7 g freeze dried soil using a mix of chloroform, methanol and citrate buffer (1:2:0.8 v/v/v). Extracted lipids were fractionated by solid phase extraction and then derivatised by mild alkaline methanolysis. The resultant fatty-acid methyl esters were analyzed by gas chromatography (Agilents, USA) and calculated as relative abundance (mol %). Fatty acids were identified by comparison of sample retention time to a standard qualitative bacterial acid methyl ester mix (Supelco) and by using gas chromatography coupled with mass spectroscopy (Agilents, USA).

PLFA nomenclature follows (Frostegård *et al.* 1993). PLFA markers were used to quantify the relative abundances (mol %) of fungi, bacteria and arbuscular mycorrhizal fungi (AMF). The ratio of fungal to bacterial PLFA was calculated as the ratio of the fungal fatty acid indicator (18:2ω6,9) to the sum of the predominantly bacterial fatty acids (15:0, i15:0, ai15:0, i16:0, ai16:0, cyc16:0, 16:1ω9, 16:1ω7cis, 16:1ω7trans, 17:0, i17:0, ai17:0, cyc17:0, 10Me17:0, cyc19:0) (Frostegård & Bååth 1996). The PLFA 16:1ω5 was not included as being from bacterial origin because it is also found in arbuscular mycorrhizal fungi (Nordby *et al.* 1981).

2.5 Statistical analysis

Treatment effects on the fungal/bacterial ratio and AMF abundance data were analysed using a three-way ANOVA. Treatment effects on plant species richness was tested using a generalised linear model with Poisson distribution. Principal component analysis (PCA) was used to visualise the variability among the measured soil variables (soil moisture content, pH and extractable nutrients) between the different treatments. Analyses were performed with R (R Development Core Team 2011), PCA was done using the ade4-package (Dray & Dufour 2007).

Permutational distance-based multivariate ANOVA (permanova)

Permutational multivariate analysis of variance (PERMANOVA: Anderson 2001) was used to determine the effect of the experimental treatments on the floristic and microbial composition of the mesocoms. This method is more appropriate than traditional methods such as MANOVA to analyze changes in composition as it can be based on any ecologically relevant distance metric and not solely on Euclidean distances (McArdle & Anderson 2001). Furthermore, *p*-values are derived from permutations of the raw data or the residuals, whereby the data do not have to conform to a multivariate normal distribution. Given that the distributions of abundances of individual species are often highly skewed or aggregated (e.g. Gaston & McArdle 1994), this broadens the applicability of PERMANOVA compared to MANOVA. We used the Bray-Curtis distance metric. Both floristic and PLFA data were log+1 transformed prior to analysis.

To complement the distance-based multivariate ANOVA tests, we used canonical analysis of principal coordinates (CAP; Anderson & Willis 2003) to visualise and interpret significant differences between treatment levels obtained from PERMANOVA. Both analyses were conducted using the vegan-package (Oksanen *et al.* 2010) in R (R Development Core Team 2011).

Co-Correspondence analysis

To compare the power of different explanatory variables in predicting floristic or microbial composition, we used direct ordination techniques, with either PLFA or vegetation composition as response variable. Depending on the explanatory data, two different techniques were used: Co-correspondence analysis (CoCa) and a predictive form of Canonical Correspondence Analysis (CCA-PLS).

Predictive co-correspondence analysis is a relatively new ordination method (see ter Braak & Schaffers (2004) and Schaffers *et al.* (2008) for a full description of the method) that can be used to quantify the strength of one composition dataset in predicting the other composition dataset. A 'leave-one-out' cross-validation is implemented whereby a regression model ('partial least squares') is calculated as many times as there are sites, each time with a different site left out. After each run, the species composition for the left-out site is predicted from its explanatory data using the obtained model parameters. This way, a predicted composition is obtained which is subsequently compared to the actual observed data. To assess the prediction accuracy we used the percentage cross-validatory fit, calculated as: $100 \times (1 - ssp_a/ssp_0)$ with ssp_a the sum of squared prediction errors of the model, and ssp_0 the sum of squared prediction errors under the null model of no relationship (site-species independence; equal relative abundances at all sites). Due to the predictive nature of the co-correspondence analysis, the prediction levels, expressed as percentages cross-validatory fit, are lower than what would be expected when using the explained variation in an explanatory method. Any prediction level greater than zero indicates that the prediction is better than what could be expected under the assumptions of the null model (CCA-PLS: ter Braak & Schaffers 2004).

When the explanatory data did not consist of composition data, predictive Canonical Correspondence Analysis (ter Braak & Schaffers 2004) was used to assess the strength of a set of predictor variables in predicting PLFA or vegetation composition. To be able to compare the results with those of CoCa, leave-one-out cross-validation was also incorporated in CCA (Simpson 2009). Four sets of predictor variables were used: the factorial design; all soil variables (nutrient concentrations, pH and soil moisture content); the sets of soil variables that were the best predictors for respectively the floristic and microbial community composition. This variable selection was performed by checking all possible subsets of 3 or more soil variables.

Both CCA-PLS and CoCA provide multidimensional solutions and in all analyses we used the number of axes at which maximum prediction accuracy was obtained.

Although the models themselves are validated implicitly (prediction level above zero), a test is still needed to judge whether differences between different models using different predictor variables are actually significant or could also be the consequence of random variation. The significance of the difference in prediction level between models was assessed using a permutation test on the prediction error sums of squares for each plot. Therefore we performed two-tailed randomization tests based on 9999 permutations.

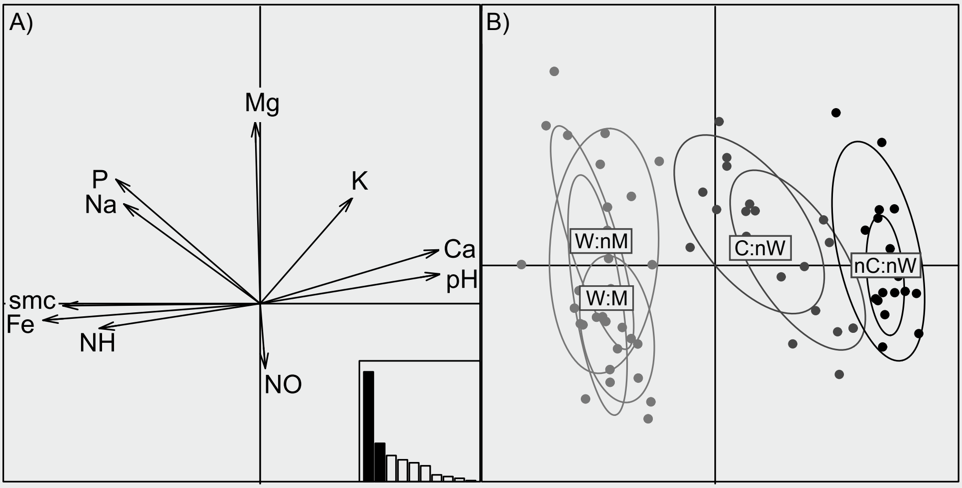
Both floristic and PLFA data were log+1 transformed prior to analysis. The CCA-PLS and CoCA analysis were carried out using the ‘cocorresp’ package of the R statistical program (Simpson 2009). The MATLAB routine of Schaffers *et al.* (2008) for cross-validation of CCA-PLS was converted to R (Mitchell *et al.* 2010). The permutation test used the ‘onet.permutation’ function within the DAAG package of the R statistical program (Maindonald & Braun 2010). Both packages are available in R freeware (R Development Core Team 2011).

→ **Fig 1.** PCA of soil variables. A) variables projections with eigenvalues diagram (F1: 44.4%, F2:15.5%), B) projections of the mesocosms grouped per treatment. (C: canopy, W: waterlogging, M: summer-mowing). (smc: soil moisture content, C: grass canopy, W: waterlogging, M: summer-mowing, n: no).

3. RESULTS

3.1 Soil variables

The variability among the measured soil variables (soil moisture content, pH and extractable nutrients) was analysed using a PCA. The first axis accounted for 44.4% of the variation (Fig. 1A), with a clear differentiation between waterlogged and non-waterlogged mesocosms. Yet, waterlogging interacted with grass canopy cover. Non-waterlogged mesocosms with grass canopy cover (C:nW) were situated intermediate between the waterlogged mesocosms and those sharing the combination of being non-waterlogged and lacking a grass canopy cover (nC:nW). Sites subjected to waterlogging had a higher soil moisture content and higher concentrations of extractable Fe, NH_4, Na and P compared to non-waterlogged sites, which showed a higher pH and higher amounts of extractable Ca and to a lesser extent K. The second axis accounted for 15.5% of the variation and did not show a clear relationship with any of the applied treatments. In the waterlogged treatment, mowing appeared to explain more variation than the light regime at inoculation, which is exactly opposite to the situation in the non-waterlogged mesocosms (Fig. 1B). The measured values of the different soil variables are summarized in Appendix B.



3.2 Floristic community composition

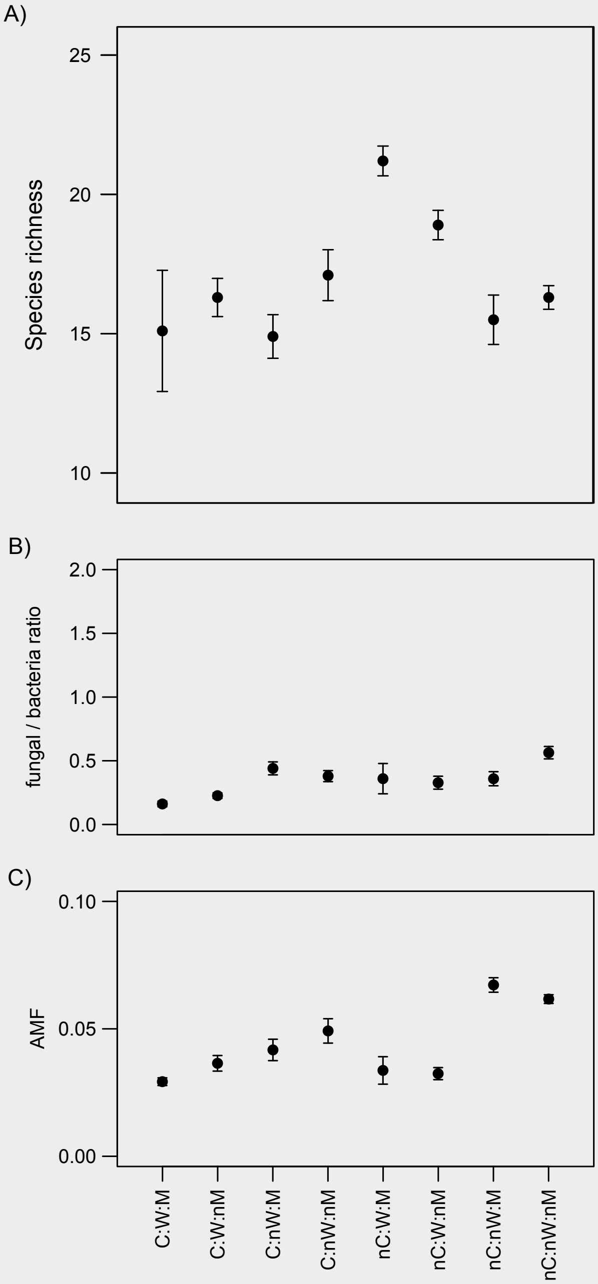
Plant species richness was only significantly affected by the interaction between waterlogging and initial vegetation cover (Z = 2.295, *p* = 0.0295; Fig. 2A).

Canonical analysis of principal coordinates (CAP) was used to find the linear combinations of principal coordinates that best discriminated experimental treatments. The resulting CAP biplot (Fig. 3A, left panel) shows how differences in floristic community composition among mesocosms were mainly determined by the main effects of the three treatments (grass canopy cover, soil waterlogging and mowing regime) and to a lesser extent by their interactions. This confirms the results of the permanova analysis, which showed that the three main treatments all had significant effects on the composition of the floristic community (Table 1A). Together, these main treatment effects accounted for 54% of the observed variation. Furthermore all possible interactions were significant explaining another 12% of the variation. Thus, in total 66% of the variation in floristic community composition could be explained by the experimental treatments, whereby grass canopy cover at sowing (27%) and waterlogging (22%) were most important.

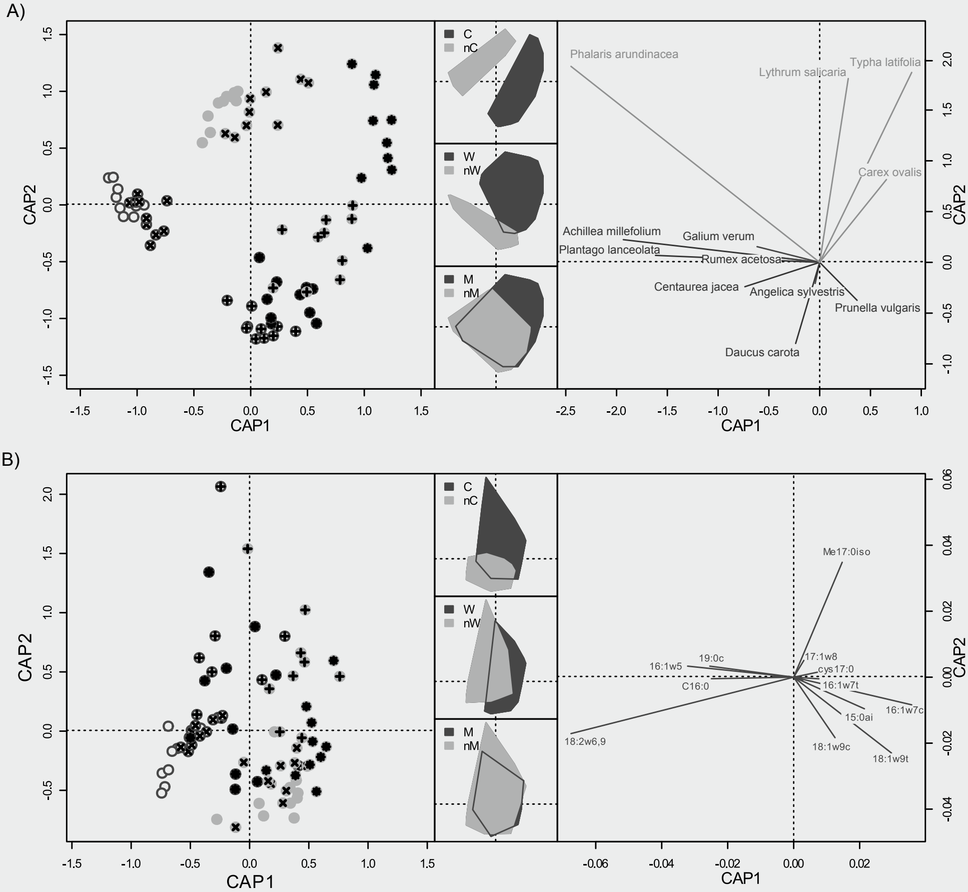
A vector overlay of all species showing a Pearson correlation r > 0.5 was added to the CAP biplot (Fig. 3A, right panel). This cut-off was used to improve visual clarity by eliminating species showing weaker relationships with the MDS axes. A distinct preference of wetland species for the waterlogged treatment was observed. Species as *Achillea millefolium* and *Plantago lanceolata* showed distinct preferences for non-waterlogged conditions without initial grass canopy sward.

3.3 Soil microbial community composition

The CAP biplot (Fig. 3B, left panel) shows how, in contrast to the floristic community composition, differences in the soil microbial community composition among mesocosms were mainly determined by only two of the experimental treatments (waterlogging and grass canopy at sowing). The results of the permanova analysis confirmed the minor effect of the different experimental treatments on the composition of the microbial community (Table 1B), accounting for only 34% of the observed variation in the soil microbial community composition, as opposed to 66% for the floristic community. The effects of both grass cano-



↑ **Fig 2.** Treatment effects on A) plant species richness, B) fungal/bacterial ratio and C) AMF abundance. Shown are mean ± SE.



↑ **Fig 2.** Constrained Analysis of Principal Coordinates using Bray-Curtis distance, highlighting the differences in a) vegetation composition and b) microbial composition among the different treatments (C: grass canopy, W: waterlogging, M: summer-mowing); Left panel shows site scores with all different treatment combinations indicated (C: ✚, W: ●, M: ×); Middle panels show the convex hulls for each of the main treatments; Right panels, Vectors represent the plant species/PLFAs showing a Pearson correlation r > 0.5 with any of the two CAP axes. Light gray: wetland species (Ellenberg-moisture values ≥ 7), Dark gray: non-wetland species (Ellenberg-moisture values < 7).

↗ **Table 1.** Treatment effects on A) floristic and B) soil microbial community composition using permutational multivariate ANOVA, with Bray-Curtis distances and log+1 transformed data; Treatment abbreviations (C: canopy, W: waterlogging, M: summer-mowing); significance levels (ns: not significant, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$). Significant effects are marked bold

A) Floristic community

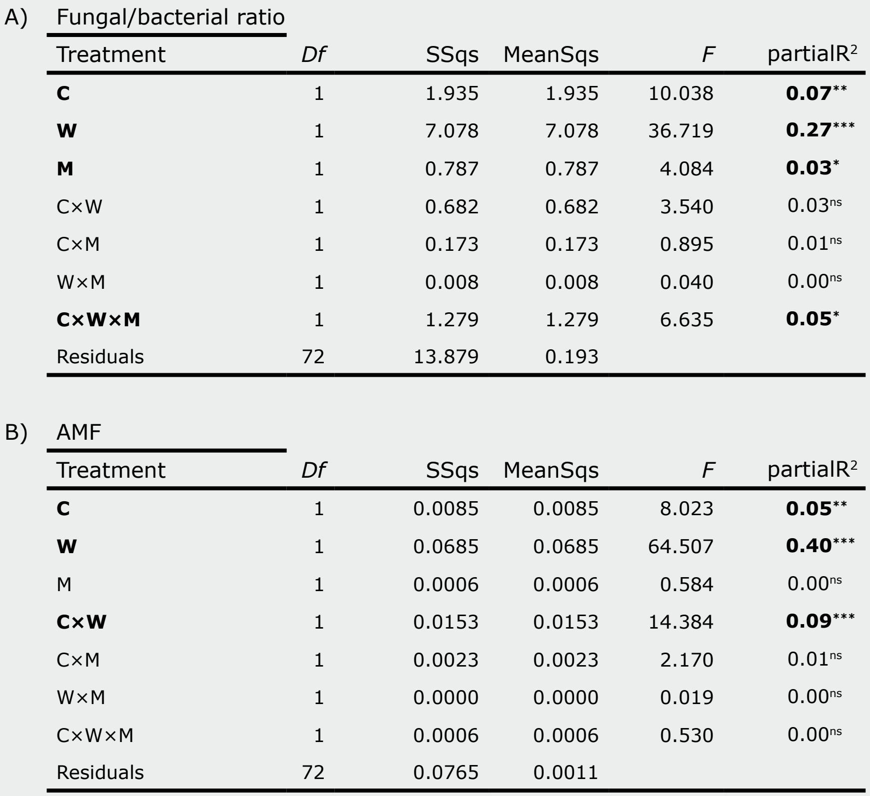
Treatment	*Df*	SSqs	MeanSqs	*F*	partialR^2
C	1	5.088	5.088	56.561	**0.27*****
W	1	4.225	4.225	46.968	**0.22*****
M	1	0.924	0.924	10.270	**0.05*****
C×W	1	0.587	0.587	6.530	**0.03*****
C×M	1	0.593	0.593	6.593	**0.03*****
W×M	1	0.530	0.530	5.888	**0.03*****
C×W×M	1	0.526	0.526	5.845	**0.03*****
Residuals	72	6.476	0.090		0.34
Total	79	18.948			1.00

B) Microbial community

Treatment	*Df*	SSqs	MeanSqs	*F*	partialR^2
C	1	0.128	0.128	8.971	**0.08*****
W	1	0.301	0.301	21.077	**0.19*****
M	1	0.029	0.029	1.999	0.02^{ns}
C×W	1	0.021	0.021	1.468	0.01^{ns}
C×M	1	0.029	0.029	2.016	0.02^{ns}
W×M	1	0.009	0.009	0.648	0.01^{ns}
C×W×M	1	0.013	0.013	0.940	0.01^{ns}
Residuals	72	1.029	0.014		0.66
Total	79	1.560			1.00

py cover and waterlogging on the composition of the soil microbial community were statistically significant. Yet, while the percentage of variation explained by waterlogging (19%) remained roughly the same as for the floristic community structure, the proportion of variation in microbial community composition accounted for by the grass canopy treatment was reduced to 8% as compared to 27% for the floristic community (Table 1).

Waterlogging had a major effect on the fungal/bacterial ratio, with on average lower ratios in waterlogged mesocosms (Table 2, Fig. 2B). Grass canopy cover interacted with waterlogging in controlling the relative abundance of AMF. In the absence of waterlogging, AMF were less abundant in mesocosms with an initial grass canopy cover than in those without (Table 2, Fig. 2C). However, in the presence of waterlogging, grass canopy cover did not affect AMF abundance.

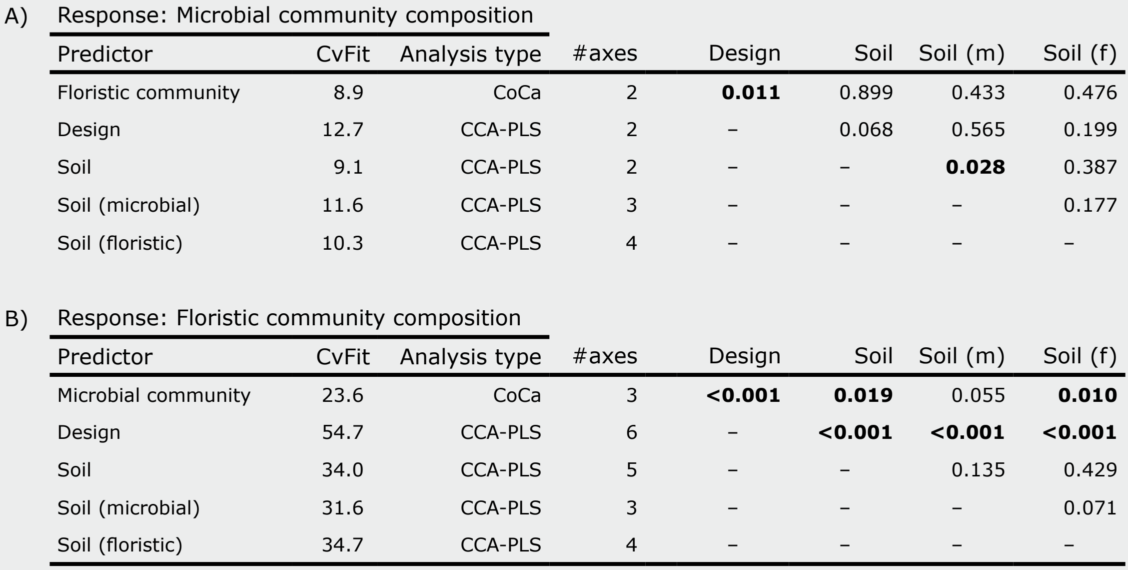


A) Fungal/bacterial ratio

Treatment	*Df*	SSqs	MeanSqs	*F*	partialR^2
C	1	1.935	1.935	10.038	**0.07****
W	1	7.078	7.078	36.719	**0.27*****
M	1	0.787	0.787	4.084	**0.03***
C×W	1	0.682	0.682	3.540	0.03^{ns}
C×M	1	0.173	0.173	0.895	0.01^{ns}
W×M	1	0.008	0.008	0.040	0.00^{ns}
C×W×M	1	1.279	1.279	6.635	**0.05***
Residuals	72	13.879	0.193		

B) AMF

Treatment	*Df*	SSqs	MeanSqs	*F*	partialR^2
C	1	0.0085	0.0085	8.023	**0.05****
W	1	0.0685	0.0685	64.507	**0.40*****
M	1	0.0006	0.0006	0.584	0.00^{ns}
C×W	1	0.0153	0.0153	14.384	**0.09*****
C×M	1	0.0023	0.0023	2.170	0.01^{ns}
W×M	1	0.0000	0.0000	0.019	0.00^{ns}
C×W×M	1	0.0006	0.0006	0.530	0.00^{ns}
Residuals	72	0.0765	0.0011		



A) Response: Microbial community composition

Predictor	CvFit	Analysis type	#axes	Design	Soil	Soil (m)	Soil (f)
Floristic community	8.9	CoCa	2	**0.011**	0.899	0.433	0.476
Design	12.7	CCA-PLS	2	–	0.068	0.565	0.199
Soil	9.1	CCA-PLS	2	–	–	**0.028**	0.387
Soil (microbial)	11.6	CCA-PLS	3	–	–	–	0.177
Soil (floristic)	10.3	CCA-PLS	4	–	–	–	–

B) Response: Floristic community composition

Predictor	CvFit	Analysis type	#axes	Design	Soil	Soil (m)	Soil (f)
Microbial community	23.6	CoCa	3	**<0.001**	**0.019**	0.055	**0.010**
Design	54.7	CCA-PLS	6	–	**<0.001**	**<0.001**	**<0.001**
Soil	34.0	CCA-PLS	5	–	–	0.135	0.429
Soil (microbial)	31.6	CCA-PLS	3	–	–	–	0.071
Soil (floristic)	34.7	CCA-PLS	4	–	–	–	–

A vector overlay of all PLFAs showing a Pearson correlation r > 0.5 was added to the CAP biplot (Fig. 3B, right panel); we used this cut-off to improve visual clarity, by eliminating PLFAs showing weaker relationships with the MDS axes. The link of fungi (18:2ω6,9) and AMF (16:1ω5) with non-waterlogged conditions is clearly confirmed.

3.4 Predicting community composition

As opposed to the above explanatory methods, direct ordination techniques were used to compare the power of different explanatory variables in predicting the floristic or microbial community composition, with either vegetation of PLFA composition as response variable. Depending on the explanatory data, two different techniques were used: Co-correspondence analysis (CoCa) and a predictive form of Canonical Correspondence Analysis (CCA-PLS). Overall, it was much more difficult to predict the composition of the soil microbial community than the composition of the floristic community. The prediction levels of the microbial community also showed less variation (8.9%–12.7%) compared with those of the floristic community (23.6%–54.7%; Table 3 – CvFit). The composition of both the microbial and the floristic community could be predicted best using the experimental treatments (waterlogging, grass canopy presence and mowing) as explanatory variable (prediction levels of, respectively, 12.7% and 54.7%; Table 3 – CvFit). As opposed to the predictive power of the experimental treatments, the predictions of the composition of one community based on the other one were much less accurate, with prediction levels for the composition of the microbial and plant community composition being only 8.9% and 23.6%, respectively.

↖ **Table 2.** Treatment effects on A) AMF and B) the fungal/bacterial ratio using three-way ANOVA; Treatment abbreviations (C: canopy, W: waterlogging, M: summer-mowing); significance levels (ns: not significant, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$). Significant effects are marked bold.

← **Table 3.** Comparision of five different predictors for A) microbial community and B) floristic community using co-correspondence analysis. Prediction levels are given as %cross-validatory fit (CvFit). Significance of the differences in prediction level among predictor variables was assessed using 9999 permutations. *P*-values are shown.

For both communities, the combination of soil variables that predicted the composition of the community most accurately was selected. This variable selection was performed by checking all possible subsets of 3 or more soil variables. In case of the soil microbial community, a combination of the extractable concentrations of Na, K, Ca, Mg and Fe predicted the composition best, while a combination of pH, soil moisture content and the extractable concentrations of Na, Ca, Mg, Fe and P did so for the floristic community. Predictions levels based upon (a subset of) soil variables were intermediate to the prediction levels based on the experimental treatments and the composition of the other community (Table 3 – CvFit).

Although the models themselves are validated implicitly (prediction level above zero), the significance of the differences in prediction levels between different models using different predictor variables has to be tested to exclude errors due to mere random variation. This was assessed using a permutation test on the prediction error sums of squares. Not all prediction levels for the different predictor variables differed significantly. For the floristic community, the prediction level of the experimental treatments differed significantly from those of the sets of soil variables and of the microbial community. The prediction levels of the different sets of soil variables did not differ mutually. Nonetheless, most of them showed significantly higher prediction levels compared to the prediction level based on the microbial community (Table 3). In the case of the microbial community, fewer differences among prediction levels were significant, as opposed to the floristic community. The experimental treatments predicted the microbial community composition significantly better than the floristic community did and only marginally better than the full set of soil variables did. The other predictor variables did not show significant differences in predictive power. Only the reduced set of soil variables which was best at predicting the microbial community, had a significantly higher predictive power than the full set (Table 3).

4. DISCUSSION

The composition of both communities was best explained by the experimental treatments (waterlogging, initial presence of vegetation and mowing). However, both communities responded slightly differently. The microbial community only showed a significant response to waterlogging and grass canopy. The vegetation on the other hand was also affected by other factors and interactions between them (Yu & Ehrenfeld 2010). However, these interactions explain much less variation than the main effects and we consider them therefore as ecologically less relevant.

Our results clearly stress the importance of soil moisture status as a major determinant of both plant and microbial community composition. Despite the fact that waterlogging forms a major stress-factor for many plant species (Ernst 1990), floristic richness is maximal in this treatment, probably due to lowered inter-specific competition for light (Kotowski *et al.* 2010). The strong effect of soil moisture status on soil microbial community structure confirms previous research. Ground water depth affects the relative abundance of several microbial groups (Balasooriya *et al.* 2008) with a reduction of both fungal/bacterial ratios and AMF after waterlogging (Mentzer *et al.* 2006, Jaatinen *et al.* 2007). Changes in the hydrologic regime affected both the structural and functional aspects of the microbial community much more than nutrient additions (Mentzer *et al.* 2006).

An initial vegetation presence is a second major determinant. Unlike waterlogging, which explained an equal amount of compositional variation in both communities, the effect of the initial grass canopy is more pronounced in the floristic than in the microbial community. Even in the third season after the onset of the experiment it still explained more than 25% of the floristic community composition and was thus even more important than waterlogging. Due to the severe light stress, seedlings were not able to establish unless mowing temporarily alleviated this stress (Kotowski *et al.* 2010). Hence, initial vegetation cover can be considered as having a large priority effect on wetland plant community assembly (Körner *et al.* 2008). This priority effect takes place above ground, but is most likely also reflected to some degree in the microbial community through e.g. altered effects of root exudates, litter quality, etc. The effect of plant species richness on soil microbial community composition is rather unclear (Gastine *et al.* 2003) and possibly depends on the functional composition of the aboveground community (Spehn *et al.* 2000, Stephan *et al.* 2000, Ladygina & Hedlund 2010, but see Marshall *et al.* 2011). Plants may also affect the soil microbial community indirectly. Several wetland species are known to increase oxygen levels in the immediate vicinity of their roots (Wießner *et al.* 2002, Colmer 2003), creating a more heterogeneous environment of oxygen-rich patches in an anaerobic environment, thus increasing the number of niches for soil micro-organisms. Also, fungi and bacteria are known to react differently to organic matter composition, fungi for instance are better at digesting organic matter with a high C:N ratio (Henriksen & Breland 1999). The limited response in the fungal/bacterial ratio in relation to initial vegetation cover that we found thus suggests the absence of large differences in C:N ratios between treatments, at least not at this initial development stage. The negative response of AMF abundance to the grass canopy cover is likely due to the lower degree of AMF colonisation in wetland monocots than in dicots (Weishampel & Bedford 2006). Moreover, the multitude of root hairs and finer branched root system of grasses is believed to make them less dependent on mycorrhizal fungi (Zhu *et al.* 2000), possibly contributing to the lower occurrence of AMF in the grass-dominated mesocosms.

Using co-correspondence analysis, the power of different sets of variables (or communities) in predicting the composition of a community was compared (ter Braak & Schaffers 2004). In our analysis, the prediction levels were markedly higher for the plant (up to 55%) than for the soil microbial community (only 13%). However, waterlogging not only has a strong effect on community composition, but also shows a strong linkage with other soil variables as revealed by the PCA. On top of its direct effects, waterlogging therefore affects community composition also indirectly through several of these soil variables. Nonetheless, in the case of the plant community, the predictive power of the applied experimental treatments was almost twice as high as that of any other combinations of soil variables. This means that the experimental design not only affected community composition through soil processes but emphasizes also the importance of aboveground processes, probably competition for light, in determining the establishment success and thus final vegetation composition.

As mentioned before, the predictability of the soil microbial community composition is rather low. Nonetheless, our prediction levels are still in the same range as those observed by Mitchell *et al.* (2010). This lower predictability could be due to several reasons. First, soil microbial communities often show a significant time-lag in response to environmental changes (Groffman *et al.* 1996, Hedlund *et al.* 2003, Habekost *et al.* 2008, Eisenhauer *et al.* 2010). A second, more technical reason could be that different techniques were used to quantify the composition of the respective communities; vegetation analyses were conducted at species level, while analyses of the soil microbial community were carried out at a higher level. Thus, even with a constant fungal/bacterial ratio there could be major shifts in species composition in both fungal and bacterial communities.

For both the vegetation and microbial communities, the composition of the other proved to be a bad predictor. Groffman *et al.* (1996) reported soil type as a more important controller of microbial biomass and activity than plant species identity. This does not agree with the findings of Mitchell *et al.* (2010) who reported that vegetation composition predicted soil microbial community composition better than soil chemistry. They considered vegetation composition as a more stable ‘summary’ of the effects of multiple drivers over time and thus as a possibly better predictor of the soil microbial community. Our results are more in line with traditional community ecology theory which puts forward abiotic conditions as main drivers of species diversity (Odum 1971, Gough *et al.* 2000).

These contrasting outcomes again confirm the difficulties in predicting aboveground consequences of belowground interactions and vice versa. Depending on the context, an organism or group of organisms on one side of the aboveground-belowground interface can exert positive, neutral, or negative effects on the other side. The nature of this context dependency is likely to be determined primarily by the spatial and temporal scale and by abiotic factors (Wardle *et al.* 2004). Hence, future research should determine how biotic relationships interact with abiotic factors in driving community composition. A better understanding of these interactions will also assist restoration ecology (Kardol & Wardle 2010).

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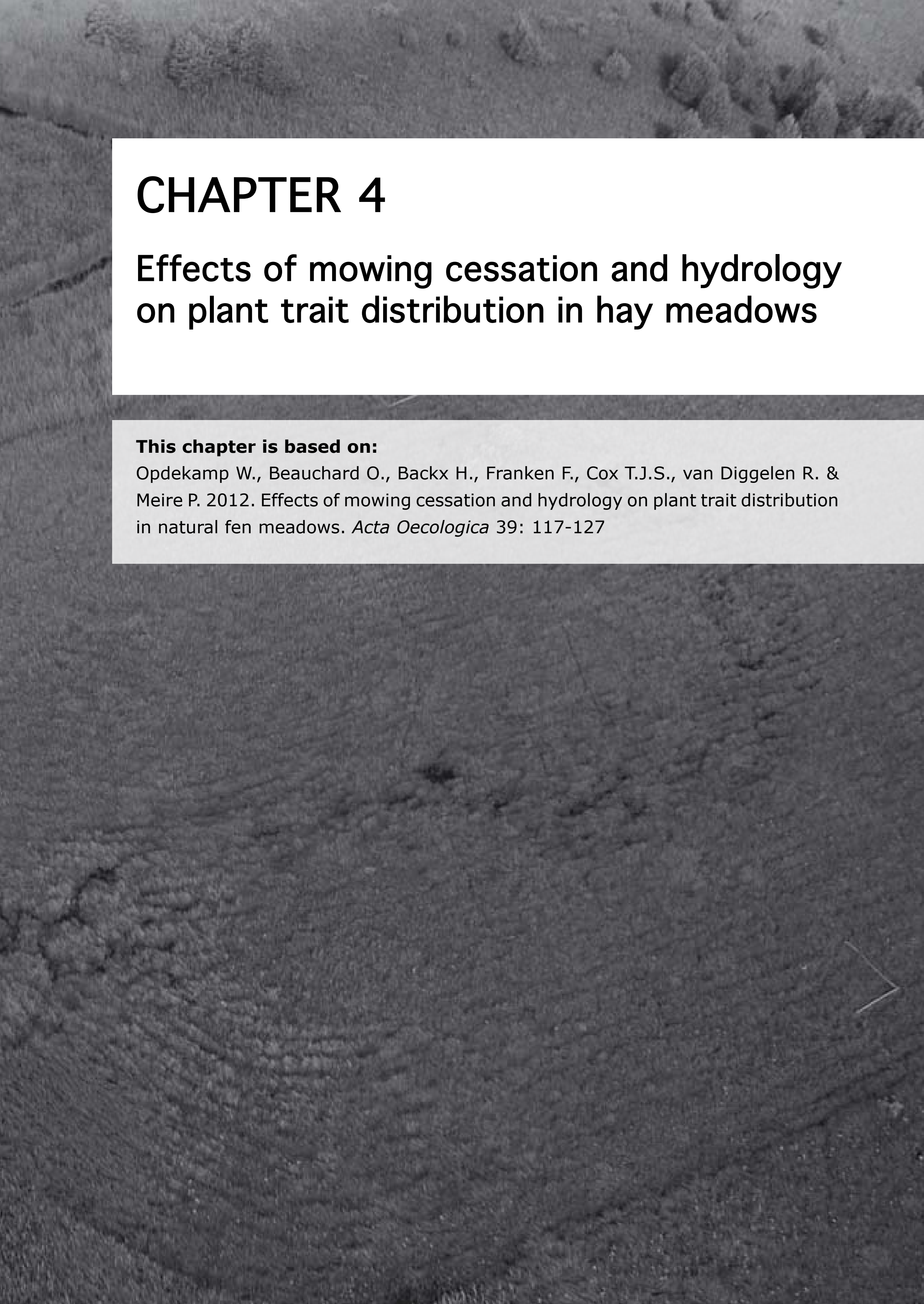
↓ **Appendix A.** Species selected for the experiments, F = Ellenberg moisture indicator (Ellenberg *et al.* 1992), SM = mass of 1000 seeds (amount introduced to each mesocosm) ; names follow Tutin *et al.* (2001).

Mesic habitat species	F	SM (g)	**Wetland species**	F	SM (g)
Achillea millefolium	4	0.17	*Carex ovalis*	7	0.49
Daucus carota	4	0.87	*Cirsium oleraceum*	7	1.90
Galium verum	4	0.54	*Eupatorium cannabinum*	7	0.22
Leucanthemum vulgare	4	0.43	*Geum rivale*	7	1.21
Angelica sylvestris	5	2.46	*Cirsium palustre*	8	1.11
Centaurea jacea	5	2.33	*Epilobium hirsutum*	8	0.11
Heracleum sphondylium	5	7.35	*Filipendula ulmaria*	8	0.60
Leontodon autumnalis	5	0.80	*Lythrum salicaria*	8	0.06
Lolium perenne	5	0.90	*Myosotis palustris*	8	0.28
Plantago lanceolata	5	2.63	*Phalaris arundinacea*	8	1.02
Poa pratensis	5	0.31	*Senecio aquaticus*	8	0.24
Prunella vulgaris	5	0.86	*Valeriana officinalis*	8	0.72
Rumex acetosa	5	0.81	*Carex acuta*	9	0.90
Alopecurus pratensis	6	0.71	*Lychnis flos-cuculi*	9	0.16
Ranunculus acris	6	1.79	*Lycopus europaeus*	9	0.27
Urtica dioica	6	0.16	*Peucedanum palustre*	9	1.35
Veronica longifolia	6	0.07	*Typha latifolia*	10	0.04

Treatment	pH		Na (µg g-1)		K (µg g-1)		Ca (µg g-1)		Mg (µg g-1)		Fe (µg g-1)		P (µg g-1)		NO (µg g-1)		NH4 (µg g-1)		SMC (%)	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
C:W:M	6.39	0.03	237	8	188	23	5175	132	473	18	2466	55	80.1	13.3	0.368	0.034	7.17	0.77	60.8	0.4
C:W:nM	6.51	0.05	249	11	235	11	5250	88	509	24	2472	24	115.7	19.5	0.336	0.002	7.97	1.15	61.3	0.8
C:nW:M	6.44	0.05	259	10	234	20	6113	152	547	14	951	92	81.1	13.8	0.353	0.031	1.10	0.61	56.5	0.5
C:nW:nM	6.56	0.05	225	9	270	19	5990	147	505	20	678	134	69.4	12.0	0.320	0.001	1.01	0.12	55.4	0.4
nC:W:M	6.34	0.03	273	11	134	10	5652	132	489	19	2598	42	91.4	16.2	0.306	0.028	5.17	0.51	61.6	0.3
nC:W:nM	6.42	0.04	315	10	189	15	5805	147	528	22	2573	76	92.2	16.2	0.397	0.043	6.09	0.97	62.0	0.7
nC:nW:M	7.07	0.03	220	6	207	17	7322	126	516	10	283	5	34.5	6.0	0.322	0.022	2.44	0.28	55.1	0.5
nC:nW:nM	6.96	0.04	220	6	273	18	7259	140	507	14	313	15	58.2	13.8	0.394	0.088	1.89	0.52	54.9	0.6

← **Appendix B.** Summary of measured soil variables in different treatments



CHAPTER 4

Effects of mowing cessation and hydrology on plant trait distribution in hay meadows

This chapter is based on:
Opdekamp W., Beauchard O., Backx H., Franken F., Cox T.J.S., van Diggelen R. & Meire P. 2012. Effects of mowing cessation and hydrology on plant trait distribution in natural fen meadows. *Acta Oecologica* 39: 117-127

ABSTRACT

Traditional grasslands are often of high conservation value, but depend on non-intensive management like mowing for their preservation. During the 20th century, traditional agricultural usage was either heavily intensified or abandoned due to socio-economic reasons. In Eastern Europe, land abandonment mainly took place in regions with qualitatively bad soils. This large scale land use change lead to secondary succession. In fens and fen meadows, this may lead to a decrease in species richness and a replacement of specialist species by more generalist ones. The main objective of the present study is to examine if and how mowing cessation interacts with hydrology in determining species and trait distribution in a fen meadow. In the Upper Course of the Biebrza National Park, Poland, we selected 15 sites along four transects, with plots in mown and abandoned parcels. In these plots we measured plant abundance, aboveground biomass and relative light intensity, while plant traits were selected from different trait databases. The relationship between these plot characteristics and the different traits was assessed using concordance analysis. Mowing cessation resulted in reduced moss cover and light availability, while vegetation height increased and higher litter deposition and tussock development were observed. This altered environment not only resulted in decreased species richness and evenness in abandoned plots but also caused shifts in plant trait distribution. Most of the significantly linked traits responded more strongly to mowing cessation than to the hydrologic gradient. Traits related to light competition, such as light requirements, plant height and shoot growth form, especially responded to mowing cessation. This stresses the importance of light competition as a major factor determining species and trait distribution in fen systems.

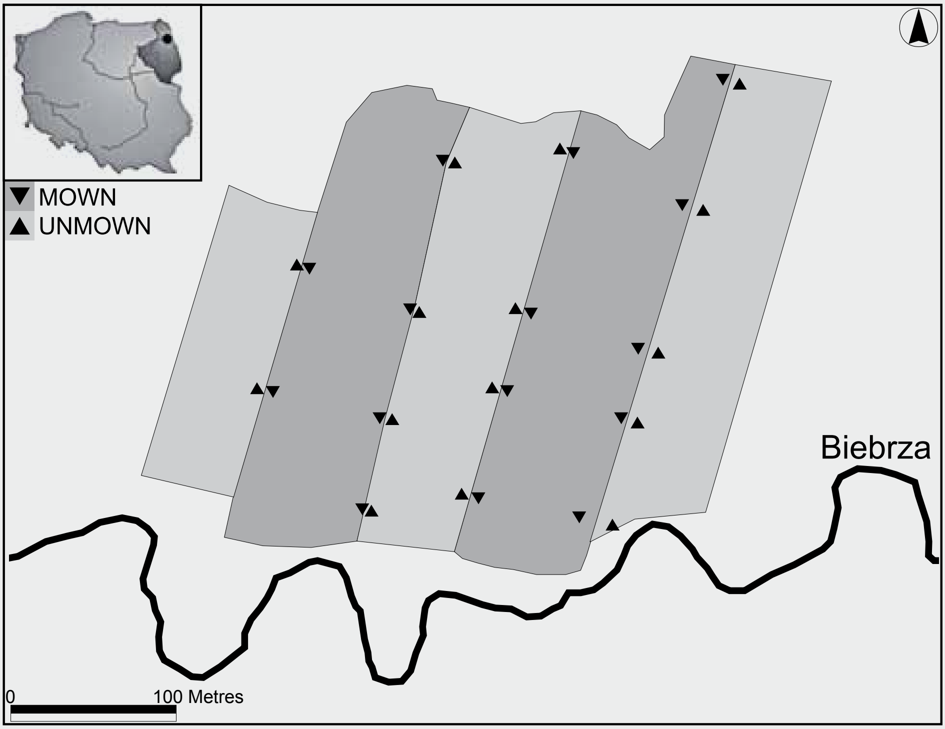
1. INTRODUCTION

In Europe, traditional grasslands (often called "semi-natural") are of high conservation value as they support many Red List species (Küster & Keenleyside 2009). Because these sites are man-made, they usually depend on non-intensive management like mowing or extensive grazing for their preservation (Bakker 1989). However, during the 20th century, the traditional agricultural usage was either heavily intensified, or abandoned due to socio-economic reasons (Poschlod *et al.* 2005). In Eastern Europe, land abandonment mainly took place in regions with qualitatively bad soils like peaty or poor moraine soils (Küster & Keenleyside 2009). This large scale land use change led to secondary succession in a range of different vegetation types. In fens and fen meadows, abandonment often results in a fast, initial development towards fallows with tall herbs and tussock-forming sedges. This phase can remain rather stable for several decades. Scrub encroachment will eventually take over resulting in the development of *Salix* or *Alnus* thickets. This succession may lead to a decrease in species richness, a replacement of specialist species by more generalist ones and an increase in productivity (Fossati & Patou 1989, Jensen & Schrautzer 1999, Billeter & Diemer 2000, Diemer *et al.* 2001, Peintinger & Bergamini 2006). These shifts are partly caused by changes in the physical environment. Litter accumulation is considered as one of the primary mechanisms responsible for a decrease in species richness and an increase of productivity in abandoned fens (Wheeler & Giller 1982, Jensen & Schrautzer 1999). It does not only result in a reduction of the amount of radiation reaching the soil surface but also in a lowered light quality as the Red:Far-Red ratio is reduced. Furthermore, it dampens the thermal amplitude in the soil and can cause an increase in phytotoxins (Facelli & Pickett 1991, Xiong & Nilsson 1999, Jensen & Gutekunst 2003). This results in decreased germination, seedling establishment, and shading out of small prostrate rosette species (Jensen & Schrautzer 1999, Stammel *et al.* 2006). Finally, bryophyte density is also severely diminished through burial by litter (Peintinger & Bergamini 2006). As a result of the increased productivity and denser canopy structure, the amount of light penetrating the lower ranges of the canopy decreases (Schrautzer & Jensen 2006). Hence, competition for light will increase which may result in a changed vegetation composition (Kotowski *et al.* 2006).

However, management is not the only driving factor determining vegetation composition in natural brook valleys. In these systems, vegetation gradients run perpendicular to the river (e.g. Pałczyński 1984). Hydrological gradients have traditionally been considered as one of the main explanatory factors for species distributions in lowland riparian systems (e.g. Wassen *et al.* 1990, Grootjans *et al.* 1996), as water regime controls nutrient availability through its effects on peat mineralization rates. The zone near the river, normally occupied by reed and tall sedges, is the most productive, due to high water level fluctuations and the input of nutrient-rich surface water. The productivity declines with increasing distance from the river. Farther from the river groundwater levels remain constantly high with little fluctuation throughout the year while the vegetation is dominated by small-sedge species and brown mosses (Kotowski & van Diggelen 2004). Kotowski *et al.* (2006) pointed out that productivity gradients and their influence on competition intensity are probably the most important factor structuring vegetation zonation in lowland riparian fens. However, these productivity gradients do not always correlate well with the hydrological gradient (Kotowski & van Diggelen 2004). This means both should be incorporated when studying changes in vegetation patterns in lowland fens, especially in areas where human management has interfered with naturally occurring patterns. Both management and hydrology are thus known to affect the vegetation composition in fens and fen meadows. However, little is known about the possible interplay between succession due to abandonment and the presence of a hydrological gradient on the species composition of these systems.

To understand the processes occurring during secondary succession, species-traits were used instead of just species identity as they reflect a species adaptation to its environment (Menezes *et al.* 2010). Hence, a trait-based approach not only allows a comparison of the same process in different vegetation types (e.g. Díaz *et al.* 2001, Lavorel & Garnier 2002, Kahmen & Poschlod 2008), but also gives insight into the mechanisms responsible for such patterns (Kahmen & Poschlod 2004) and allows predictions about possible future changes.

The main scope of the present paper is to examine if and how mowing cessation interacts with hydrology in determining species and trait distribution in a fen meadow. Specifically, we try to answer the following questions: Do the changes in the physical environment, caused by mowing cessation, only affect floristic diversity or does mowing cessation also lead to a shift in functional traits? If so, to what extent do management and the hydrologic gradient drive these changes in trait distribution?



↑ **Fig 1.** Location of the study site. Parcels 1, 3 and 5 are abandoned, parcels 2 and 4 are mown annually. The black triangles indicate the sampling points, respectively in managed (▲) and unmanaged parcels (▼).

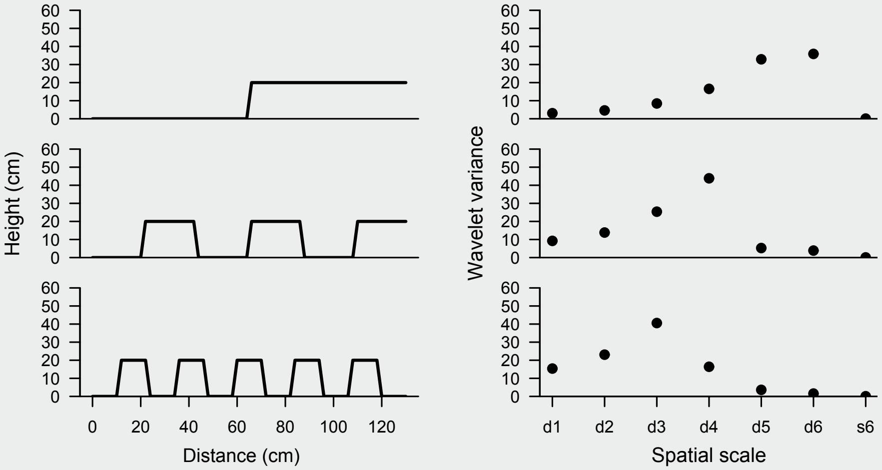
2. METHODS

2.1 Site description

In the Biebrza National Park in Eastern Poland (Fig. 1), unreclaimed low productive fen meadows have been mown traditionally for hay. However, abandonment began in the 1970's. Between 1981 and 1983, ca. 30000ha was still mown annually (Banaszuk 1994). Today less than 5000ha of meadows within the park boundaries is still mown or grazed. In the Upper Basin, the Biebrza river valley is only a few hundred metres wide compared to several kilometres in the Lower Basin. As it is only slightly drained, the characteristic zonation pattern of plant communities from the riverbed to the valley margins is still present. A more detailed description of the Biebrza valley can be found in Wassen *et al.* (2006).

Our study plots are located near Rogozyn (N53°42'16", E23°26'30") in the Upper Basin (Fig. 1). Here, the river is only 5–6 meter wide. Discharges in early summer (June) range from 0.12 to 0.98 m^3 s^{-1} (De Doncker 2009). The soil consists of sandy/loamy peat (1–2 m deep, underlain by fluvial and eolian sand). The (former) hay meadows are bordered by a sandy moraine in the north (130m ASL) and by the river Biebrza in the south (waterlevel app. 119.5m ASL). Altitudes are expressed relative to the Polish height system Kronstadt 86. Throughout the season the water table fluctuates between -30 and +10 cm. Shallow flooding occurs near the river during late winter-early spring (Struyf *et al.* 2009).

In this study we selected five adjacent parcels with an average size of 60m × 270m. Until the late eighties to early nineties all parcels were subjected to an annual mowing regime (end of June–July). However, annual mowing has been abandoned between 1991 and 1995 (pers. comm. local farmers) in three parcels, which led to secondary succession and the development of tussocks (up to 30cm high). Along four transects perpendicular to the river, we selected 15 sites with a plot both in the mown and abandoned parcel (Fig. 1). Within a transect, the height difference between sites near the moraine and sites near the river is approximately 1m. In order to reduce side effects, plots (2m × 2m) were located 5m from the parcel border.



↑ **Fig 2.** Three idealised topographic situations and their corresponding wavelet variance. The standard deviation of the height is exactly the same in the three situations (SD = 10 cm), and therefore can not be used to distinguish between them. The wavelet variance distributes the variance over different length scales d1-d6, and peaks at lower values when characteristic length scales of topographic variations are smaller. The weighted average of these wavelet variances ('Tuss') is used as an indicator for characteristic tussock size in a plot.

2.2 Plot characteristics

The relative influence of different water sources (lateral-flowing water from the valley flank, discharging ground water, flooding river water and rainwater) changes from valley flank to brook, causing a zonation in hydrological features such as wetness characteristics and water composition. Since most soil process (e.g. mineralization rate) are governed by hydrological conditions (e.g. soil moisture, pH) the same zonation is expressed in soil nutrient status (Grootjans & Tenklooster 1980, Succow 1988). Therefore we used the distance of a plot to the river ('Dist'), determined as the shortest possible distance between a plot and the river, as a summarising proxy for the hydrological connectivity of a site with the river.

At each plot the topography of the soil surface was measured three times using a pinframe (Kent & Coker 1995). Soil height was measured every 2 cm along this frame (1.4 m – 70 points per frame). To quantify the typical spatial scale of elevated elements along the pinframe, we calculated the wavelet variance of the obtained topographical profiles (Percival 1995). The wavelet variance attributes variability in the profile to different (spatial) scales. The weighted average (‘Tuss’) of these variances is a measure for the typical tussock size per plot (Fig. 2). The variance (‘Topv’) of the topography was also calculated per plot. At each contact with the soil, all vascular plant species touching the pin were recorded. Species abundance in a plot was determined as the number of contacts summed over the three frames. Aboveground biomass in all subplots was harvested in a 0.5m × 0.5m square and sorted into living vascular plants (‘Biom’), mosses (‘Moss’) and dead biomass (‘Dead’). Samples were dried for 48h at 70 °C, weighed and expressed per m². Before harvesting we measured a vertical light profile in every plot. Relative Light Intensity (RLI) was measured at 5 cm height increments by horizontally inserting the 84cm long rod of an Accupar LP-80 (Decagon Devices, Inc.; Pulman; USA), starting from groundlevel with a reference probe well above the vegetation.

For each profile we determined the height at which RLI was equal to 30% (‘L30’) and to 90% (‘L90’) of full sunlight. For most phanerogams the light saturation point is reached at approximately 30% RLI, as a further increase in light does not increase photosynthesis (Fliervoet 1984, Larcher 2003). The RLI of 90% was measured to indicate the height of the vegetation and is easier to define than the 100% point, because of the open structure of the upper-most layers in some plots.

Species abundance data and topographical data were collected in 2005, 2006 and 2008 after which the data of the three years was combined for analysis. Plot characteristics related to biomass (‘Biom’, ‘Dead’, ‘Moss’) and light availability (‘L30’, ‘L90’) were collected in 2008. To reduce the effect of extreme values, part of the plot characteristics (‘Topv’, ‘Moss’, ‘L30’, ‘L90’) were square root transformed before analysis. An overview of the plot characteristics can be found in Table 1.

↓ **Table 1.** Description of the different plot characteristics

Plot characteristic	Description	Units
L30	Height at which RLI is 30%	cm
L90	Height at which RLI is 90%	cm
Tuss	Size of tussocks, based on wavelet decomposition (see text)	—
Topv	Variance of height	—
Dist	Distance of site to river	m
Biom	Standing biomass	g/m^2
Dead	Dead biomass	g/m^2
Moss	Moss biomass	g/m^2

↓ **Table 2.** Trait description. Given are abbreviations of traits and possible attributes, both followed by their description and the data source (Ellenberg et al. 1992, BioBase: CBS 2003, BiolFlor: Kuhn *et al.* 2004, CloPla: Klimešová & Klimeš 2006, LEDA: Kleyer *et al.* 2008, SID: Liu *et al.* 2008)

Trait		Attribute		Source
Bs	(Budbank – Seasonality)	Bn	: belowground, none	CloPla
		Bs	: belowground, seasonal	
		Bp	: belowground, perennial	
		Gn	: groundlevel, none	
		Gs	: groundlevel, seasonal	
		Gp	: groundlevel, perennial	
		An	: aboveground, none	
		As	: aboveground, seasonal	
Bv	(Budbank - Vertical distribution)	b0	: belowground, none	CloPla
		b1	: belowground, 1-10 buds	
		b2	: belowground, >10 buds	
		g0	: groundlevel, none	
		g1	: groundlevel, 1-10 buds	
		g2	: groundlevel, >10 buds	
		a0	: aboveground, none	
		a1	: aboveground, 1-10 buds	
		a2	: aboveground, >10 buds	
Cgo	(Clonal growth organs)	0	: no clonal growth organ	CloPla
		1	: above ground “runners”	
		2	: above ground “plant parts”	
		3	: below ground “runners”	
		4	: below ground “plant parts”	
Flod	(Duration of flowering)	1	: short, 1-2 months	Biolflor
		2	: medium, 3-4 months	
		3	: long, >4 months	
Flos	(Start of flowering)	1	: January-April	Biolflor
		2	: May-June	
		3	: July-September	

Height	(Maximal plant height)	1	: 0-25%, [0.0-0.4m]	LEDA
		2	: 25-50%,]0.4m-0.7m]	(Rothmaler only)
		3	: 50-75%,]0.7m-1.0m]	
		4	: 75%-100%,]1.0m-4.0m]	
Lat	(Lateral spread)	0	: no lateral spread	CloPla
		1	: <0.01 m	
		2	: 0.01-0.25 m	
		3	: >0.25 m	
Ld	(Leaf distribution along stem)	g	: rosette plant	Biolflor
		h	: hemirosette plant	
		r	: erosulate plant	
Lightmax	(Maximal light requirement)	FL	: full light	BioBase
		L	: light	
		LS	: light shadow	
Lightmin	(Minimal light requirement)	L	: light	BioBase
		LS	: light shadow	
		HS	: half shadow	
		S	: shadow	
Lightran	(Range in light requirement)	0	: no difference between min and max	BioBase
		1	: 1 category difference	
		2	: 2 categories difference	
		3	: 3 categories difference	
		4	: 4 categories difference	
Moist	(Moisture indicator)	4	: in-between 3 and 5	Ellenberg
		5	: on fresh soils, i.e. under intermediate conditions	
		6	: in-between 5 and 7	
		7	: on moist soils which do not dry out	
		8	: in-between 7 and 9	
		9	: on wet, often not well aerated soils	
		10	: on frequently inundated soils	
		×	: indifferent	
Rep	(Reproduction type)	s	: by seed/by spore	Biolflor
		ssv	: mostly by seed, rarely vegetatively	
		sv	: by seed and vegetatively	
		svv	: mostly vegetatively, rarely by seed	
		v	: vegetatively	
Seed	(Seed mass)	1	: 0-25%, [0-0.17 mg]	LEDA/SID
		2	: 25-50%,]0.17-0.60 mg]	
		3	: 50-75%,]0.60-1.06 mg]	
		4	: 75%-100%,]1.06-17.73 mg]	
Sgf	(Shoot growth form)	l	: liana	LEDA
		p	: prostrate stem	
		a	: ascending to prostrate stem	
		e	: erect stem	
Sla	(Specific leaf area)	1	: 0-25%, [0.0-18.5 mm^2 g^{-1}]	LEDA
		2	: 25-50%,]18.5g-22.3 mm^2 g^{-1}]	
		3	: 50-75%,]22.3g-27.8 mm^2 g^{-1}]	
		4	: 75%-100%,]27.8g-74.2 mm^2 g^{-1}]	

2.3 Plant traits

Traits which were predicted to respond to secondary succession or to the hydrological gradient (e.g. McIntyre *et al.* 1999, Weiher *et al.* 1999) were extracted from several plant trait databases (Table 2). Where a database contained several entries per species-trait combination the following aggregation scheme was used. For categorical traits, we discerned two cases: (1) entries to different trait-attributes are likely (e.g. a species can have several clonal growth organs), and (2) species have only one possible entry per trait (traits: Flod, Flos, Ld, Lightmax, Lightmin, Lightran, Moist, Rep). In the first case (traits: Bs, Bv, Cgo, Sgf) the number of entries per trait-attribute was expressed relatively. For the trait "clonal growth organs" this would e.g. look like: "no clonal growth organs" [1,0,0,0,0], "aboveground runners" [0,1,0,0,0], "aboveground plant parts"/"belowground runners" [0,0,1,1,0], etc. In case more than one entry per species-trait combination is found in a database, these values are weighted by the number of entries per trait-attribute and expressed relatively to the total number of entries for that trait.

For numerical traits (traits: Height, Lat, Seed, Sla), the median value of all possible entries was used. Afterwards, these numerical traits were turned into categorical traits by taking the 25th, 50^{th} and 75th percentiles of all median values and assigning a score of 1–4 to each species.

2.4 Data analysis

General community characteristics

The evenness of species abundance was measured using Pielou's evenness index. This index performs well on three main features that an evenness index should meet: dependence with richness, symmetry criteria and variation range (Beisel *et al.* 2003). A linear mixed-effect model was run to test the effects of distance to the river and mowing cessation on the different environmental characteristics as well as on both diversity indices (species richness and Pielou's evenness index). Site was added as a random factor.

Relationships between plot characteristics and plant traits

A multivariate approach was conducted to identify the plant traits significantly related to the environmental conditions. Environmental data were arranged in a sites × plot characteristics table. For each trait, the distribution of trait-attributes per site (sites × trait-attributes table) was obtained by multiplying the sites × species-abundance table with the species × trait-attribute table. To counter possible richness-effects, species abundance data were expressed relatively per site.

The relationship between the plot characteristics and the different traits was explored by concordance analysis (Lafosse & Hanafi 1997). This multivariate technique is a derived version of co-inertia analysis (Dolédec & Chessel 1994) which searches for linear combinations (independent axes) between the variables of two tables sharing the same statistical units (e.g. sites); most of the time this approach is conducted by canonical correspondence analysis (CCA), despite the fact that this latter is generally not optimal (Dray *et al.* 2003). Co-inertia axes are constructed by considering both tables as two multidimensional clouds of matched points so that eigenvalues (standard deviations of the axes) maximize the concordance of the two multidimensional configurations. The strength of the relationship between both tables is quantified through *Rv*-coefficients, equivalent to a simple correlation coefficient between two variables (Robert & Escoufier 1976). Their significance is tested with a procedure based on random permutations (here 9999) of the lines of the paired tables (Heo & Gabriel 1998). Graphically, besides variable projections on the axes, co-inertia analysis allows the simultaneous projection of both point clouds in order to appraise the strength of their fitting (similarly to predicted and observed values in a simple regression). In the present situation, concordance analysis extends this approach to one reference table (plot characteristics) and *K* tables with plant traits, similarly to the extension of simple to multiple regression analysis. Each axis is constituted of an independent part of the co-variation between the plot characteristics and each of the plant traits. These are weighted to their respective inertia to avoid possible over-influence of traits having a larger number of variables (trait-attributes). Prior to concordance analysis, a first step consists of selecting the trait-tables correlated significantly to the plot characteristics table by performing *K* co-inertia analyses. This step ensures the visualisation of the dimensionality of the link (covariance) between each plant trait and the plot characteristics (eigenvalues diagram). Then, concordance analysis proceeds with the coordination of the *K* links. For each trait-table it provides a ratio of the link in its independent co-inertia analysis and in the coordinated analysis. Hence, the contribution among the biological traits can be compared. For application examples in ecology, see Bozec *et al.* (2005) and Townsend *et al.* (2003).

Concordance analysis was carried out with ADE-4 (Université de Lyon 1, Villeurbanne, France, http://pbil.univ-lyon1.fr/ADE-4-old/ADE-4.html). Other statistical analyses were performed with R 2.10.1 (R version 2.10.1, R Foundation for Statistical Computing, Vienna, Austria). Wavelet variance was calculated from the maximal overlap discrete wavelet transformation using the waveslim-package (R package version 1.6.3, Brandon Whitcher). Linear mixed-effect models were run using the nlme-function in the nlme-package (R package version 3.1-97, nlme: Linear and Nonlinear Mixed Effects Models). Multivariate graphs were made using the ade4-package (R packages version 1.4-16, Biometry and Evolutionary Biology Lab - University Lyon 1, Villeurbanne, France).

3. RESULTS

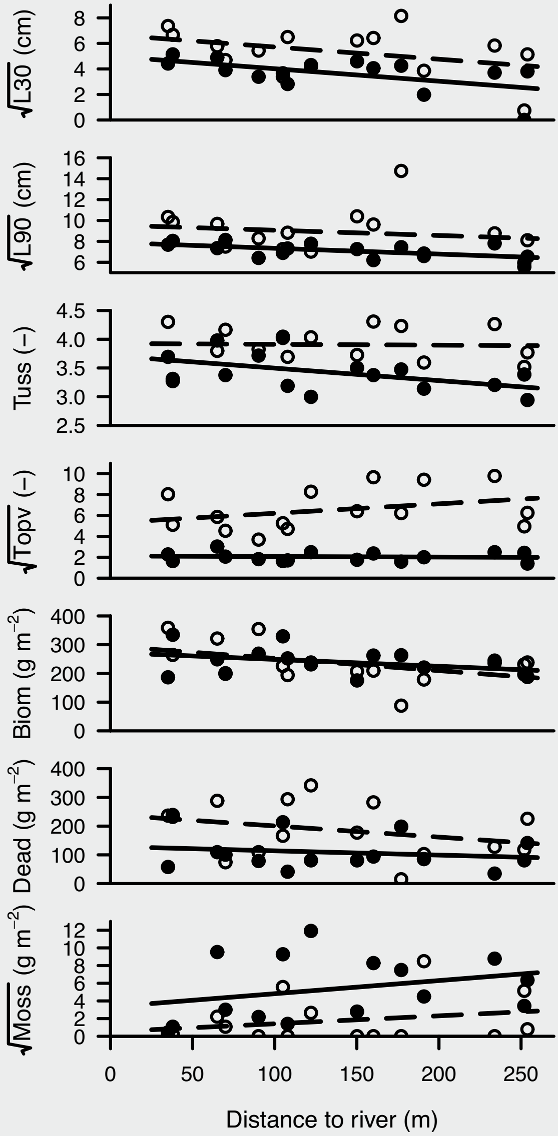
3.1 Plot characteristics

Except for the aboveground biomass of vascular plants ('Biom'), no plot characteristics showed a significant relationship with the distance of a plot to the river ('Dist'). Biom was highest near the river and decreased further away. Light availability is highest in mown parcels as both 'L30' and 'L90' are significantly higher in abandoned plots. Tussock size ('Tuss'), topographical variability ('Topv') and the amount of litter ('Dead') are significantly higher in abandoned plots. However, moss cover ('Moss') is significantly higher in mown plots (Fig. 3, Table 3).

The projection of the plot characteristics on the factorial plane of the concordance analysis is displayed in Fig. 4A (left panel). The first axis was characterised by positive covariances of vegetation height ('L90'), overall topographic variability ('Topv'), tussock size ('Tuss') and lowered light availability (high values for 'L30'), and to a lesser extent also ('Dead'), opposed to high values for moss coverage ('Moss'). The projection of the site scores for the plot characteristics forms the reference pattern (Fig. 4A, right panel). This reference pattern coincided with the difference in management regime whereby mowing cessation resulted in increased values for 'Tuss', 'Topv', 'L30' and 'L90' while 'Moss' was highest in managed sites. The second axis was mainly characterized by a positive covariance of the distance to the river ('Dist'), partly covariant with 'Topv' and 'Moss', and opposed to biomass ('Biom').

3.2 Species richness and evenness

Distance to the river did not significantly influence species richness nor evenness. However, mowing cessation strongly determined both species richness and evenness (Table 3B). Highest species richness was achieved in managed sites (Fig. 5A). Furthermore, Pielou's evenness index indicated that species abundances were more evenly distributed in managed than in unmanaged sites (Fig. 5B); see Appendix A for mean abundances of observed species.



↑ **Fig 3.** Relationship between different plot characteristics and distance of plot to the Biebrza river, for mown (●,solid line) and abandoned (○,dashed line) plots. Summarised results of the linear model can be found in Table 3. For description of plot characteristics, see Table 1.

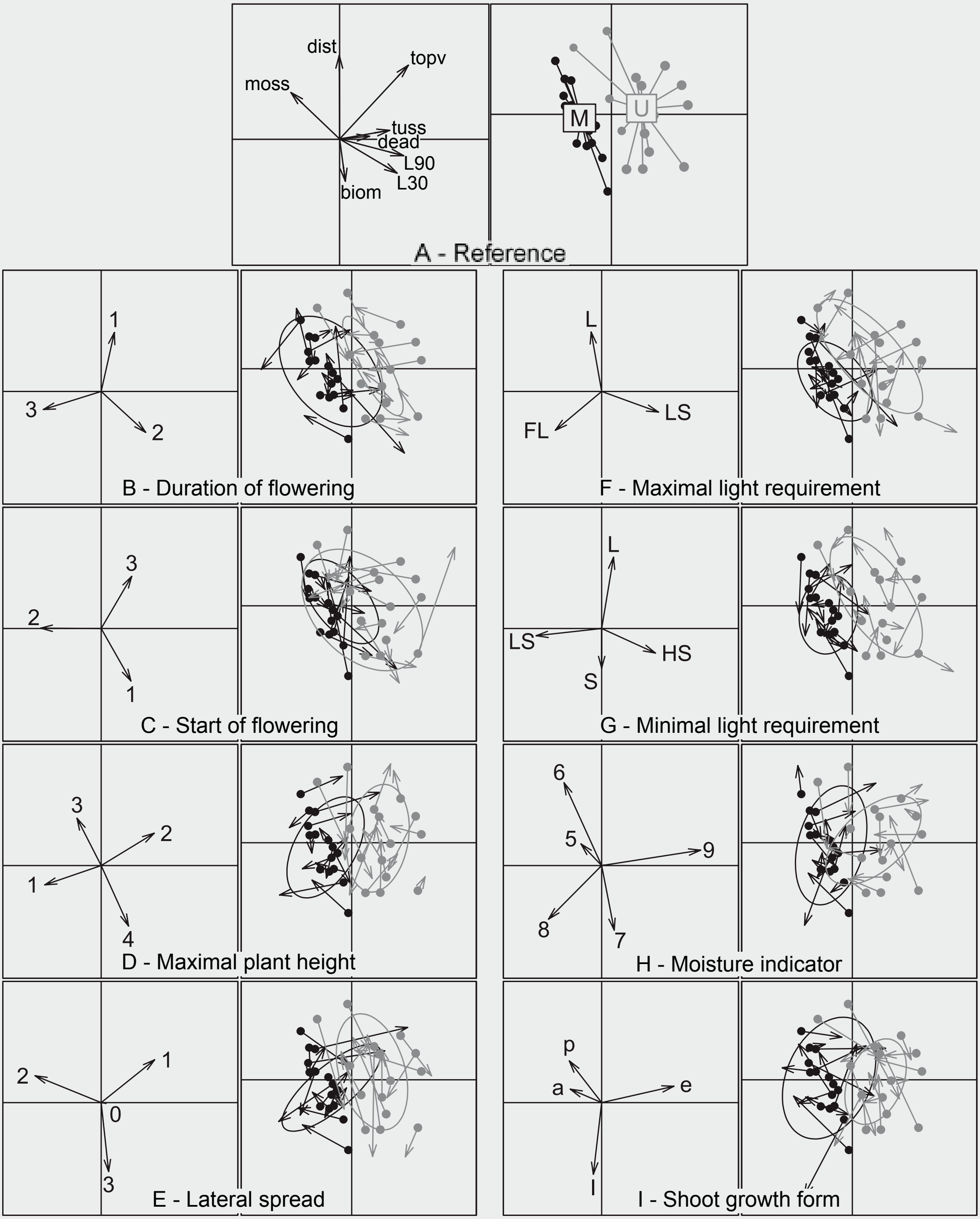
↓ **Table 3** Summarised results of the linear model with plot distance and management as explanatory variables and A) plot characteristics and B) diversity indices as response variable. Significant effects are marked in bold; results for A) and B) are respectively shown in Fig. 2 and Fig. 4; see Table 1 for abbreviations and descriptions of plot characteristics.

A)

Plot characteristic	Source	F-value	*p*-value
L30	**Management**	**28.159**	**<0.001**
	Distance	4.103	0.064
	Management × Distance	0.002	0.964
L90	**Management**	**10.680**	**0.006**
	Distance	1.440	0.252
	Management × Distance	0.005	0.943
Tuss	**Management**	**23.145**	**<0.001**
	Distance	1.807	0.202
	Management × Distance	2.017	0.179
Topv	**Management**	**92.731**	**<0.001**
	Distance	1.140	0.305
	Management × Distance	2.121	0.169
Biom	Management	0.029	0.868
	Distance	**5.362**	**0.038**
	Management × Distance	0.423	0.527
Dead	**Management**	**7.164**	**0.019**
	Distance	1.723	0.212
	Management × Distance	0.344	0.567
Moss	**Management**	**11.372**	**0.005**
	Distance	1.873	0.194
	Management × Distance	0.151	0.704

B)

Diversity index	Source	F-value	*p*-value
Species richness	**Management**	**7.8067**	**0.015**
	Distance	0.8909	0.362
	Management × Distance	0.0524	0.823
Pielou’s Eveness Index	**Management**	**31.3978**	**<0.001**
	Distance	1.1231	0.309
	Management × Distance	1.3154	0.272


A - Reference

B - Duration of flowering

C - Start of flowering

D - Maximal plant height

E - Lateral spread

F - Maximal light requirement

G - Minimal light requirement

H - Moisture indicator

I - Shoot growth form

3.3 Plant functional traits

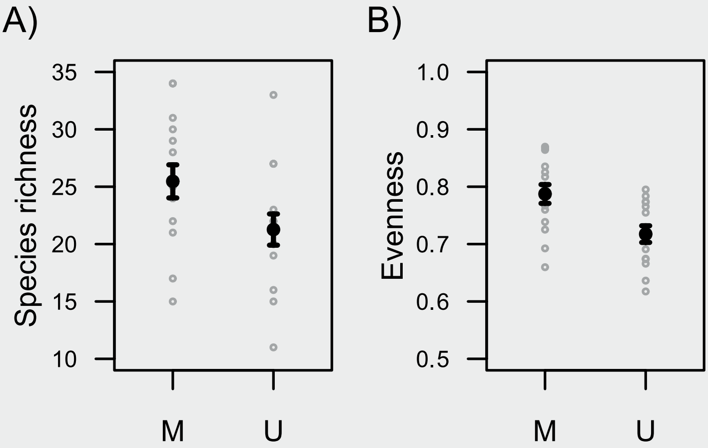
Only half of the assembled traits were significantly correlated with the plot characteristics, as illustrated by the significance of the *Rv*-coefficients between each of the trait-tables (sites × trait-attributes) and the plot characteristics table (sites × variables) (Table 4). In the separate co-inertia analyses, these significantly linked traits exhibited a one- or two-dimensional pattern encompassing most of the common information with the plot characteristics (Fig. 6). The strength of the relationships between plot characteristics and plant traits in the concordance analysis is given as the percentage of the total variance of the separate co-inertia analyses encompassed in the first two axes of the concordance analysis (Table 4, *Ratio*). This ratio differed markedly among traits as it ranged from 0.64 for ‘start of the flowering period’ (‘Flos’) up to 0.97 for the ‘minimal light requirement’ (‘Lightmin’).

The reference pattern (Fig. 4A) engendered by the plot characteristics displays a clear management effect on the first axis and a lesser effect of distance to the river on the second axis. The patterns induced by the traits returned a variable reproducibility of the reference site ordination (Fig. 4B to I, right panel). Concordance between the reference and trait patterns was assessed by Pearson’s correlations between the projections of the sites based on the plot characteristics and based on the traits. Cor1 and Cor2 give the Pearson’s correlation coefficients for respectively the 1st and 2nd axis of the concordance analysis (Table 4; *Cor1* and *Cor2*). All these traits were significantly linked to

← **Fig 4.** Concordance analysis. A) Covariances of plot characteristics (left) and consequent reference ordination of the sites (right); ●, managed sites; ●, unmanaged sites. B – I) Trait modalities (left) and related site ordination projected on the reference site projection (right); points indicate reference ordination (in A); arrow tips indicate site positions induced by trait-attributes; arrow lengths indicate lack of fitting between reference positions and the considered trait (assessed by Cor1 and Cor2 in Table 3); trait- related positions (arrow tips) are grouped per treatment (ellipses). For ’Moisture indicator’, attributes ‘4’, ‘10’ and ‘X’ are not shown as they hardly derive from the origin.

the plot characteristics on the first axis. However, this proved not to be the case for the second axis where only 'Flod', 'Height', 'Lightmin' and 'Moist' were significantly linked. The major part of the variance in most traits was projected on the first axis of the factorial plane, except for flowering duration ('Flod') where most variance was projected on the second axis.

Flowering duration was the only trait of which the distribution was primarily determined by the distance to the river. Short-flowering species (1–2 months) reached higher abundances further away from the river while the opposite is true for species flowering medium long (3–4 months). Management abandonment resulted in a slightly decreased abundance of long-flowering species. Overall, species starting to flower in May–June are most abundant. Yet, in unmanaged sites more variation in the start of the flowering period was observed (Fig. 4C, right panel). The smallest species (<0.4m) showed a clear preference for managed plots while the highest plants were most abundant in unmanaged sites close to the river. Species with very limited lateral spread were more characteristic of unmanaged sites while plants with moderate to strong lateral spread were more abundant at mown sites near by the river (low fitting of the site projections on the 1st axis). Abandonment was linked to a shift in plant light requirements. In managed plots, species with high maximal light requirements (FL) and rather high minimal light requirements (LS) were more abundant while abandonment resulted in an increase of more shade-tolerant species. However, as shown by the smaller deviations of the site projections (Fig. 4F, right panel), high maximal light requirements were more concordant with the plot characteristics in mown sites. Plants with the highest minimal light requirements (L) reached higher abundances with increasing distance to the river (Fig. 4G). Abandonment lead to increased abundances of species typical for wet, badly aerated soils. Along the second axis, species preferring moist to wet conditions affiliated with sites situated close to the river while species linked to slightly drier conditions are situated further away. Overall, species with an erect stem were most abundant. Yet, shoot growth form attributes were more variable in managed plots with slightly higher abundances of species with an ascending or an ascending to prostrate stem.



↑ **Fig 5.** A) Species richness per plot (paired-t = 2.89, df = 14, p-value = 0.01); B) Pielou's evenness index (paired-t = 5.54, df = 14, p-value = 0.00) for mown (M) and unmown (U) sites. Shown are mean±SE

↑ **Fig 6.** Eigenvalue diagram with the relative inertia for each axis of the separate co- inertia analyses, expressing the dimensionality of the links between the different significantly linked traits and the plot characteristics. For abbreviation of the traits, see Table 2.

↓ **Table 4** Statistics of concordance analysis. Rv-coefficients between each of the trait-tables (sites × trait-attributes) and the plot characteristics table (sites × variables) are equivalent to the ordinary correlation coefficient between two variables; *p*-values are the estimated probabilities equal to the frequency of random values higher than the observed Rv. Significant traits are marked in bold; Ratio returns the strength of the link between each trait-table and the plot characteristics in the concordance analysis as the percentage of the total variance of the separate co-inertia analyses, encompassed in the first two axes of the concordance analysis; Cor1 and Cor2 give the Pearson's correlation coefficient between the projections of the sites based on the plot characteristics and based on the traits, respectively for the 1st and 2nd axis of the concordance analysis; significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; for trait abbreviations, see Table 2.

Trait	***Rv***	***p*-value**	Ratio	Cor1	Cor2
Bs	0.17	0.060	–	–	–
Bv	0.12	0.193	–	–	–
Cgo	0.13	0.150	–	–	–
Flod	**0.20**	**0.021**	**0.91**	**0.57****	**0.58*****
Flos	**0.23**	**0.008**	**0.60**	**0.43***	**0.33**^{ns}
Height	**0.39**	**<0.001**	**0.94**	**0.70*****	**0.40***
Lat	**0.25**	**0.006**	**0.82**	**0.51****	**0.14**^{ns}
Ld	0.02	0.948	–	–	–
Lightmax	**0.29**	**0.001**	**0.86**	**0.63*****	**0.26**^{ns}
Lightmin	**0.51**	**<0.001**	**0.96**	**0.80*****	**0.55****
Lightran	0.17	0.934	–	–	–
Moist	**0.41**	**<0.001**	**0.93**	**0.69*****	**0.59*****
Rep	0.04	0.931	–	–	–
Seed	0.14	0.171	–	–	–
Sgf	**0.24**	**0.006**	**0.72**	**0.46****	**0.30**^{ns}
Sla	0.15	0.090	–	–	–

4. DISCUSSION

4.1 Plot characteristics

In our study, secondary succession resulted in an altered environment. Microtopography at the plot level increased after mowing cessation due to the development of tussocks (mainly of *Carex cespitosa* and *Carex appropinquata*). Preiss (1982), Fossati & Patou (1989) and Diemer *et al.* (2001) also concluded that tussock-forming perennial graminoids appear to benefit strongly from mowing cessation whereas rhizomatous graminoid species (*Carex nigra*, *Carex panicea*, *Eriophorum angustifolium*) tended to decline. Brzosko (2001) observed a significant increase in tussock size and age structure of *C. cespitosa* when comparing the same site 15 and 25 years after abandonment.

Contrary to the observations of Diemer *et al.* (2001) and Schrautzer & Jensen (2006) we observed no increase in standing crop following mowing cessation. This could partly be due to our early sampling date at the beginning of July which is before the peak of standing crop is attained. Nevertheless, Preiss (1982) also did not find a significant increase in standing crop after management cessation. The increased productivity closer to the river coincides well with the pattern observed by Wassen *et al.* (1990) and Kotowski *et al.* (2006) and resulted in slightly lower light availability closer to the river.

Despite the lack of an effect of abandonment on aboveground biomass, we observed an increased canopy height in abandoned plots which is in accordance to Diemer *et al.* (2001) who found almost a twofold increase in vegetation height. The light saturation point was also reached at higher elevations in the canopy of abandoned plots. This reduction in light availability confirms the results of Schrautzer & Jensen (2006) who found a significant decrease in RLI in abandoned plots. However, in our case RLI was not as closely linked to aboveground biomass as observed by Schrautzer & Jensen (2006) and Kotowski & van Diggelen (2004).

Litter accumulation is a well-known consequence of abandonment (Ellenberg 1986). The values we obtained (a two-fold increase) are, however, less than those observed in other studies on abandonment in fen systems. Diemer *et al.* (2001) noted a 15-fold increase in litter mass in abandoned fens. A significant decrease in moss cover was observed in abandoned sites which is probably a consequence of the reduced light availability and increased litter coverage (Peintinger & Bergamini 2006).

4.2 Species richness and evenness

Mowing cessation appeared to be a key factor in determining species richness and evenness. Fossati & Patou (1989) observed a significant species loss after abandonment of a floodplain. Jensen (1997) found that species richness and evenness decreased after abandonment of previously mown species rich wet meadows while Diemer *et al.* (2001) observed a similar pattern in calcareous fens. Despite the higher standing crop near the river we found no significant relation between the distance to the river and species richness as found by Kotowski *et al.* (2006).

4.3 Plant functional traits

The first axis of the reference pattern of the concordance analysis can be considered as management related, while the second axis is primarily dominated by the distance-productivity gradient. Of the eight traits which are significantly related to the reference pattern, only four show a significant correlation between the projections of the sites on the second axis of the reference pattern and their projections on the second axis of the different trait-tables. Furthermore, only flowering duration shows a higher correlation coefficient for the second than for the first axes. This emphasizes the dominant effect of management cessation compared to the hydrological gradient in determining the functional profile of the sites considered in this study. Fossati & Patou (1989) also concluded that mowing is the most important factor affecting vegetation composition in a floodplain. Tasser & Tappeiner (2002) found that vegetation distribution is mainly a consequence of historical and actual land use while natural site conditions only played a subordinate role. In the present study, the dominant role of the management regime over the hydrologic gradient is confirmed by the pattern shown by the moisture indicator as mowing cessation lead to increased abundances of tussock-forming sedges which normally have a preference for wet or badly aerated soils (Ellenberg *et al.* 1992). Yet, the distribution of the trait-attributes of the moisture indicator was also affected by the hydrological gradient. Hence, the pattern which could be expected under natural conditions is partly disturbed by the past and present management.

The reduced light availability due to mowing cessation appears to play an important role in determining the trait distribution. This is reflected in the pattern shown by the light requirements where species with higher light requirements are more abundant in mown or less productive sites. Furthermore, plant height, related to competitive ability (Westoby 1998), showed a high correlation with the reference structure. In dense vegetation PAR decreases with increasing depth in the canopy (Kotowski & van Diggelen 2004). Therefore, plants with a large maximal size can position their leaves in light saturated canopy parts and outshade others. Therefore, the number of small-growing species declines after management abandonment (Schrautzer & Jensen 2006). However, some species are able to counter the effects of reduced light availability to some extent through phenotypic plasticity, e.g. Billeter *et al.* (2003) observed increased shoot height of *Carex davalliana* in abandoned sites.

Finally, species with a non-erect stem show a reduced light harvesting efficiency compared to those with an erect stem. Decreased abundances of prostrate species after abandonment have been reported by Preiss (1982) and Jensen (1997). Billeter & Diemer (2000) on the other hand did not observe a clear decline of *Tofieldia calyculata*, a prostrate clonal species, after abandonment. Nevertheless, it shifted more towards vegetative reproduction.

During grassland succession, species able to spread vegetatively often show increased abundances (e.g. Kahmen & Poschlod 2004). Conversely, we observed an increase of species with very limited lateral spread following abandonment. This is probably due to the increased abundances of caespitose species like *C. appropinquata* and *C. cespitosa*.

Less variation in flowering onset was observed in mown compared to unmanaged sites. This can be due to several reasons. First of all, mowing is believed to favour species flowering earlier in the season (van Diggelen *et al.* 2005), while species in the abandoned parcels are released from the selection pressure to finish seed production by the end of July. In these parcels it can also be beneficial for subordinate plants to start flowering well before the peak of biomass is attained. Most species need sufficient light for the induction of flowering and seed production (Larcher 2003). In our study, species flowering for 3–4 months appeared to be more abundant near the river. Their prolonged flowering period might allow them to cope better with flooding disturbance, as they are able to repeat seed production under more favourable conditions. However, care should be taken when interpreting these latter two relationships as plants can show considerable plasticity in flowering onset and duration depending on climatic conditions (Weiher *et al.* 1999).

Several studies have reported successful attempts to restore fen meadows through the reintroduction of an annual mowing regime (e.g. Güsewell *et al.* 1998, Billeter *et al.* 2007). However, the tussock development observed in our study plots could seriously hamper such attempts. In case mowing management cannot be applied annually, mowing every few years might keep tussock development at a low level. As biomass harvested from low-input high-diversity (LIHD) grasslands can be used for biofuel production (Tilman *et al.* 2006), the reinstallation of an annual mowing regime and subsequent conversion of the harvest to fuel might simultaneously fulfil our energy demands and contribute to biodiversity conservation (Palmer 2006).

4.4 Conclusions

Management abandonment proved to be a bigger driving factor than the distance from the river in determining the distribution of both species and traits. Mowing cessation resulted in increased tussock size and litter cover while light availability decreased significantly. These changes in the physical environment not only lead to reduced floristic species richness and evenness, but also caused shifts in plant trait distribution. Traits related to a plant's ability to compete for light showed the biggest response to mowing cessation. Our results thus confirm the earlier findings of Kotowski & van Diggelen (2004) and Schrautzer & Jensen (2006) who stressed that light competition is a major factor determining species zonation in fen systems.

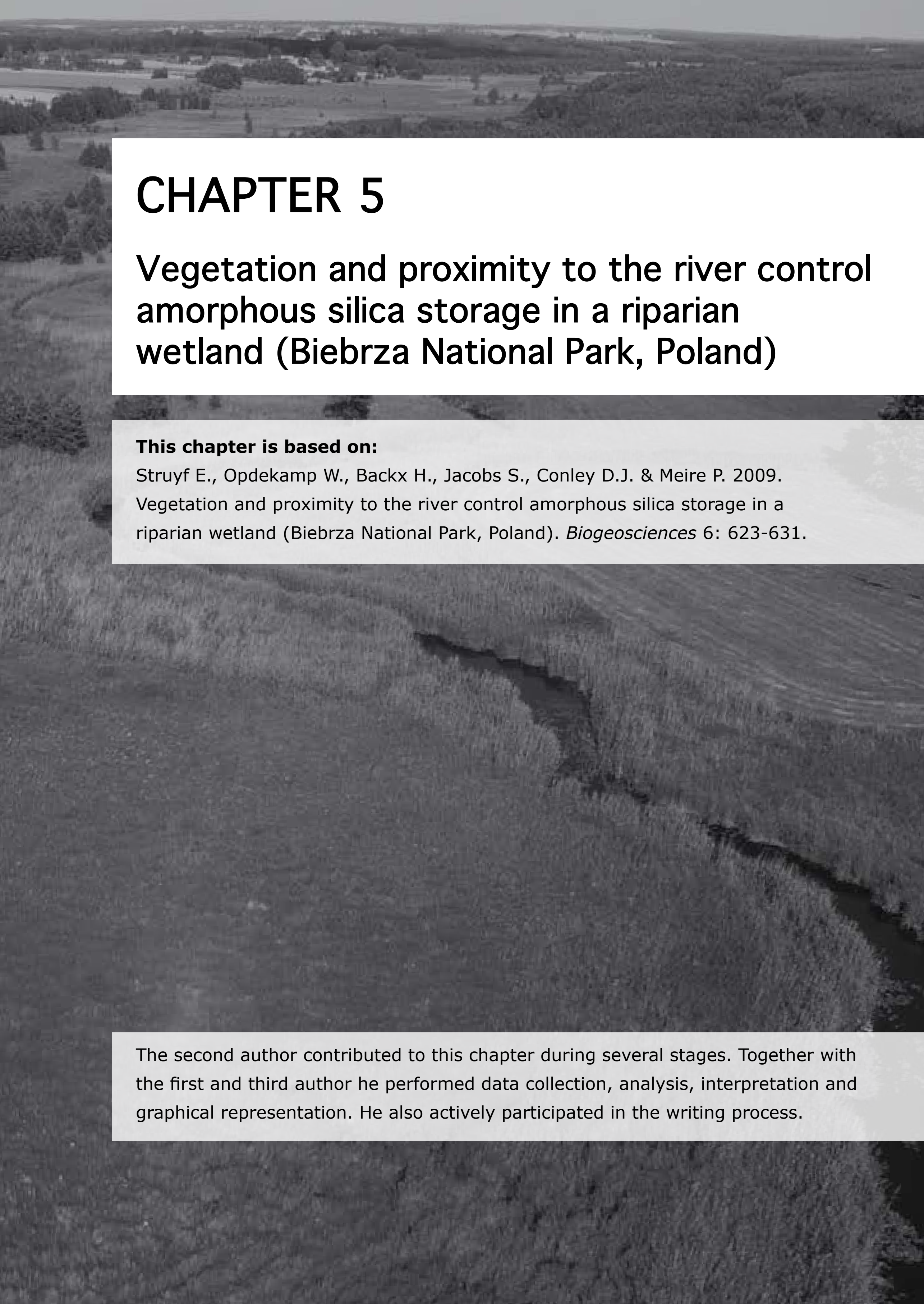
ACKNOWLEDGEMENTS

We want to thank the Board of the Biebrza National Park for their permission to work in the area. Tomek Okruszko and the whole SGGW crew are acknowledged for the coordination of the joint measuring campaigns. Finally we want to thank the students who helped during the measuring campaigns.

↓ **Appendix A.** Recorded species. % indicates the number of hits per species for managed (M) and abandoned (U), divided by the total number of hits for mown (M) and abandoned (U). Species names follow Tutin *et al.* (2001).

Name	M	U	Name	M	U
Agrostis capillaris	0.33%	0.11%	*Mentha arvensis*	0.05%	0.00%
Angelica sylvestris	0.00%	0.17%	*Menyanthes trifoliata*	0.98%	0.14%
Anthoxanthum odoratum	0.04%	0.00%	*Myosotis scorpioides*	0.98%	0.59%
Calamagrostis stricta	0.22%	0.01%	*Parnassia palustris*	0.00%	0.03%
Caltha palustris	0.55%	0.35%	*Pedicularis palustris*	0.03%	0.00%
Cardamine pratensis	0.10%	0.14%	*Peucedanum palustre*	0.00%	0.74%
Carex acutiformis	0.72%	0.00%	*Phalaris arundinacea*	0.00%	0.24%
Carex appropinquata	9.18%	22.75%	*Phleum pratense*	0.01%	0.00%
Carex cespitosa	8.51%	17.99%	*Phragmites australis*	0.00%	0.55%
Carex diandra	0.31%	0.00%	*Plantago media*	0.01%	0.00%
Carex lasiocarpa	0.00%	0.30%	*Poa palustris*	0.00%	0.10%
Carex nigra	15.89%	8.05%	*Poa pratensis*	4.51%	2.14%
Carex panicea	5.46%	0.96%	*Poa trivialis*	0.61%	1.52%
Carex rostrata	1.30%	0.07%	*Polygonum bistorta*	1.74%	2.46%
Cirsium palustre	0.00%	0.18%	*Potentilla anserina*	0.00%	0.01%
Cirsium rivulare	0.00%	0.30%	*Potentilla palustris*	4.29%	3.74%
Crepis paludosa	0.47%	0.04%	*Ranunculus acris*	3.54%	1.36%
Dactylorhiza incarnata	0.14%	0.04%	*Ranunculus lingua*	0.00%	1.15%
Deschampsia cespitosa	0.17%	0.61%	*Ranunculus repens*	3.60%	0.37%
Epilobium palustre	0.31%	0.40%	*Rhinanthus angustifolius*	0.21%	0.00%
Epilobium tetragonum	0.03%	0.03%	*Rumex acetosa*	1.13%	0.24%

Equisetum fluviatile	0.46%	0.30%
Equisetum palustre	0.30%	0.59%
Eriophorum angustifolium	0.74%	0.04%
Festuca arundinacea	0.02%	0.10%
Festuca pratensis	0.17%	0.13%
Festuca rubra	7.16%	3.54%
Filipendula ulmaria	5.04%	6.01%
Galium palustre	5.71%	0.88%
Galium uliginosum	2.31%	0.64%
Geum rivale	3.48%	7.48%
Hieracium caespitosum	0.00%	0.01%
Juncus articulatus	0.30%	0.01%
Lathyrus palustris	0.94%	0.50%
Lathyrus pratensis	0.00%	0.18%
Linum catharticum	0.01%	0.00%
Lychnis flos-cuculi	0.74%	0.18%
Lycopus europaeus	0.00%	0.28%
Lysimachia thyrsiflora	0.01%	0.23%
Lysimachia vulgaris	0.52%	2.08%
Lythrum salicaria	0.08%	0.24%
Mentha aquatica	0.89%	0.06%
Sagina procumbens	0.07%	0.00%
Salix cinerea	0.04%	0.25%
Salix pentandra	0.00%	0.04%
Scutellaria galericulata	0.01%	0.50%
Senecio paludosus	0.03%	0.25%
Sonchus palustris	0.20%	0.00%
Stachys palustris	0.00%	1.05%
Stellaria longifolia	0.05%	0.40%
Stellaria palustris	0.04%	0.03%
Taraxacum officinale agg.	0.20%	0.00%
Thelypteris palustris	0.01%	0.00%
Trifolium repens	1.98%	0.01%
Triglochin palustris	0.03%	0.00%
Trisetum flavescens	0.00%	0.07%
Urtica dioica	0.00%	0.48%
Valeriana officinalis	0.89%	0.74%
Veronica longifolia	0.46%	0.82%
Veronica serpyllifolia	0.01%	0.00%
Viburnum opulus	0.00%	0.21%
Vicia cracca	1.22%	0.76%
Viola epipsila	0.45%	2.99%



CHAPTER 5

Vegetation and proximity to the river control amorphous silica storage in a riparian wetland (Biebrza National Park, Poland)

This chapter is based on:
Struyf E., Opdekamp W., Backx H., Jacobs S., Conley D.J. & Meire P. 2009. Vegetation and proximity to the river control amorphous silica storage in a riparian wetland (Biebrza National Park, Poland). *Biogeosciences* 6: 623-631.

The second author contributed to this chapter during several stages. Together with the first and third author he performed data collection, analysis, interpretation and graphical representation. He also actively participated in the writing process.

ABSTRACT

Wetlands can modify and control nutrient fluxes between terrestrial and aquatic ecosystems, yet little is known of their potential as biological buffers and sinks in the biogeochemical silica cycle. We investigated the storage of amorphous silica (ASi) in a central-European riparian wetland. The variation in storage of ASi in the soil of an undisturbed wetland was significantly controlled by two factors: dominance of sedges and grasses and distance to the river (combined $R^2 = 78\%$). Highest ASi storage was found near the river and in sites with a dominance of grasses and sedges, plants which are well known to accumulate ASi. The management practice of mowing reduced the amount of variation attributed to both factors ($R^2 = 51\%$). Although ASi concentration in soils were low (between 0.1 and 1% of soil dry weight), ASi controlled the availability of dissolved silica (DSi) in the porewater, and thus potentially the exchange of DSi with the nearby river system through both diffusive and advective fluxes. A depth gradient in ASi concentrations, with lower ASi in the deeper layers, indicates dissolution. Our results show that storage and recycling of ASi in wetland ecosystems can differ significantly on small spatial scales. Human management interferes with the natural control mechanisms. Our study demonstrates that wetlands have the potential to modify the fluxes of both DSi and ASi along the land-ocean continuum and supports the hypothesis that wetlands are important ecosystems in the biogeochemical cycling of silica.

1. INTRODUCTION

Biologically controlled uptake and storage of silica and its recycling in terrestrial ecosystems is a significant component of the global biogeochemical silica cycle (Conley 2002). It forms an essential buffer between mineral weathering, the ultimate source of dissolved silica (DSi), and the eventual export to the aquatic environment. Biogenic amorphous silica (ASi) stored in soils as plant phytoliths and diatoms, is several orders of magnitude more soluble than mineral silicates (Farmer *et al.* 2005) and the recycling of ASi exhibits an important control on terrestrial DSi export (e.g. Derry *et al.* 2005, Fulweiler & Nixon 2005, Sommer *et al.* 2006). DSi mobilization in the terrestrial environment is the primary silica source for aquatic and eventually coastal and oceanic environments (Van Cappellen 2003), where it is an essential nutrient for diatoms (*Bacillarophyceae*). The availability of DSi relative to other nutrients can influence the occurrence of harmful algal blooms (e.g. Smayda 1997). In addition, the uptake of DSi and burial of diatoms on the ocean floor is an important sink for atmospheric carbon on biological time scales (Dugdale *et al.* 1995). Moreover, terrestrial mineral silicate weathering is an essential controlling mechanism for atmospheric CO_2 concentrations on geological timescales (Berner *et al.* 1983). Understanding the land-based cycling of silica is essential in understanding carbon biogeochemistry and eutrophication.

Yet, the biotic control of land-based silica fluxes is far from understood. Quantifying the ecosystem biotic component is one of the main challenges in understanding the biogeochemical cycling of silica. Research in terrestrial ecosystems has largely focused on forested ecosystems, where phytolith dissolution has been identified as the primary source for exported DSi (Bartoli 1983, Farmer *et al.* 2005). More recently, a literature overview suggested that wetlands have the potential to exhibit a profound influence on terrestrial silica transport and storage (Struyf & Conley 2009). Wetlands can modify nutrient fluxes along the aquatic continuum through transformation and storage. Silica rich biomass, together with the frequent occurrence of diatoms and sponges in wetlands, provides the potential for the accumulation of ASi rich soils (Clarke 2003, Struyf *et al.* 2005). Struyf & Conley (2009) hypothesized that a trade-off between the storage of ASi in wetland soils and the export of DSi from wetlands depends on hydrological and vegetation characteristics. However, little research has actually focused on the factors controlling accumulation and recycling of ASi in wetlands that could verify this hypothesis. By contrast, the importance of wetlands in other nutrient cycles (N and P) as sinks and processors has been intensively studied (e.g. Güsewell & Koerselman 2002, Gribsholt *et al.* 2005), highlighting the role of riparian wetlands in reducing excessive N and P inputs to aquatic ecosystems (e.g. Hattermann *et al.* 2004, Olde Venterink *et al.* 2006). Silica cycling related research in wetlands has focused on tidal marshes (Struyf & Conley 2009), which are characterized by low water residence times and frequent flooding leading to the rapid recycling of buried ASi (Struyf *et al.* 2006), and with increasing sedimentation resulting in more efficient burial of ASi (Struyf *et al.* 2007a). The marsh grass *Phragmites australis* is a key participant in the uptake of DSi, its transformation to ASi and recycling in tidal marshes (Struyf *et al.* 2007b). Research in tidal wetlands clearly shows the importance of hydrological connectivity and vegetation in wetland silica biogeochemistry.

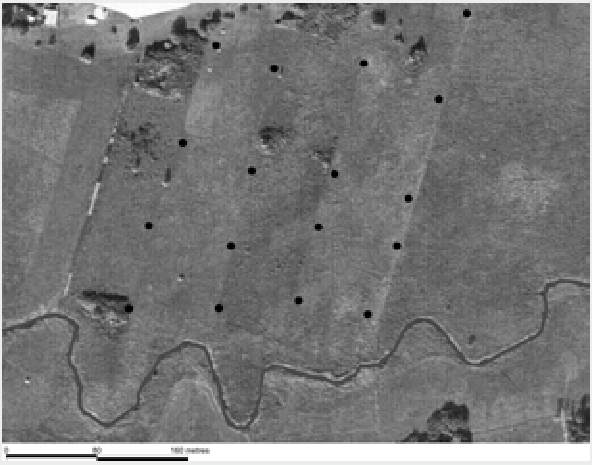
We have examined the storage of ASi in a central-European riparian wetland in Poland, characterized by a dominance of sedge and grass vegetation. A number of wetland plants are important accumulators of ASi (e.g. Lanning & Eleuterius 1983, Struyf *et al.* 2005), but *Cyperaceae* (sedges) and *Poaceae* (grasses) are the most efficient accumulators. Wetland grasses and sedges on average contain 0.5-4% of Si in their tissues, while exceptionally high values up to 20% have been recorded (Struyf & Conley 2009). We sampled for ASi along gradients in distance to the river (as a proxy for hydrology and river connectivity), in the dominance of grasses and sedges and at both managed (annual mowing) and unmanaged sites. Porewater DSi concentrations were sampled as an indicator of recycling of ASi. Our sampling was aimed to test the hypothesis by Struyf & Conley (2009) that silica storage and recycling depends on river connectivity and vegetation. Our data show that river distance and vegetation can explain most of the observed variation in ASi storage, with human management (mowing) interfering with and reducing these control mechanisms.

2. MATERIALS AND METHODS

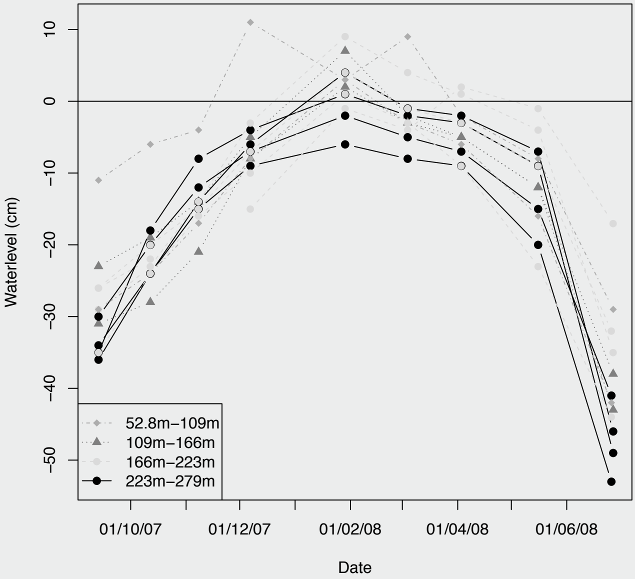
2.1 Sampling site

The Biebrza River valley (Fig. 1), located in NE-Poland (22°30′-23°60′E, 53°30′-53°75′N) harbours one of the best preserved wetlands in Central-Europe with few draining and land reclamation activities. A regular pattern of marsh and fen communities arranged parallel to the river attests to its pristine character. The river drains a catchment area of ca. 7000km^2. The area has a temperate continental climate with a mean annual gross precipitation of 583mm and a mean annual temperature of 6.8 °C. The Biebrza River valley (ca. 900 km^2) is bordered by moraines and outwash plains and can be divided in three sub-basins, the Lower, Middle and Upper Basin. The relatively narrow Upper Basin (1-3 km wide) is bordered by an outwash plain in the northwest and delimited by moraines and moraine islands composed of loamy sand and gravel in the south. A detailed description of the Biebrza River valley can be found in Wassen *et al.* (2006).

Our study site near Rogożyn (Fig. 1) in the Upper Basin consists of several parcels, differing in management practices and vegetation type. Samples for soil ASi and porewater DSi were taken at 17 sampling sites along 4 gradients perpendicular to the Biebrza River channel (Fig. 1) at the end of June 2007. The sampling distance (lowest distance perpendicular to the river) from the river was used in all statistical analysis as a proxy for hydrological connectivity to



↑ **Fig. 1** Sampling schedule at Rogozyn with all 17 sampling sites.



↑ **Fig. 2** Groundwater at the Rogozyn in function of distance from the river, as sampled at 10 locations. Positive numbers indicate groundwater level above soil surface.

the river. At each sampling site, both managed and unmanaged vegetation was sampled. The vegetation at unmanaged sites has not been mown for at least 10 years prior to sampling, while managed sites are annually mown. The sites are characterized by gradients in dominance of sedges and grasses (Fig. 4A). A summary of the dominant plant genera (all species with 10% or more coverage) at every site is provided in Table 1, as well as the total grass and sedge dominance. The depth of the groundwater table at different distances from the river sampled from September 2007 to June 2008 is provided in Fig. 2.

2.2 ASi concentrations

2 cores (Ø 2 cm) of 30 cm were taken at every site in June 2007 and in total 68 cores were taken. Every core was subdivided into three sections of 10 cm (0-10 cm, 10-20 cm and 20-30 cm) and every depth section was analysed for ASi concentration. ASi was determined by alkaline extraction of 30 mg of freeze-dried

Managed vegetation

Plot	Distance from river (m)	P+C Dominance (%)	Dominant genera
1	234	83	*Carex* (66%), *Festuca* (16%)
2	150	30	*Carex* (25%), *Geum* (18%), *Filipendula* (15%)
3	122	89	*Carex* (82%)
4	65	54	*Carex* (45%), *Menyanthes* (10%)
5	252	60	*Carex* (29%), *Festuca* (22%), *Ranunculus* (18%)
6	191	80	*Carex* (57%), *Festuca* 22 (%)
7	108	61	*Carex* (44%), *Geum* (13%), *Festuca* (9%)
8	70	61	*Carex* (43%), *Festuca* (16%), *Filipendula* (9%)
9	35	60	*Carex* (58%), Potentilla (8%), *Menyanthes* (8%)
10	262	44	*Carex* (28%), *Festuca* (10%), *Ranunculus* (9%)
11	177	42	*Carex* (41%), *Ranunculus* (14%), *Filipendula* (11%)
12	105	49	*Carex* (43%), *Galium* (10%), *Ranunculus* (10%)
13	48	35	*Carex* (18%), *Ranunculus* (15%), *Poa* (13%)
14	254	53	*Carex* (41%), *Galium* (13%), *Festuca* (8%)
15	160	48	*Carex* (29%), *Galium* (15%), *Poa* (11%)
16	90	53	*Carex* (39%), *Galium* (13%), *Poa* (10%)
17	38	41	*Carex* (35%), *Galium* (14%), *Trifolium*(9%)

Unmanaged vegetation

Plot	Distance from river (m)	P+C Dominance (%)	Dominant genera
1	234	78	*Carex* (74%)
2	150	62	*Carex* (49%), *Filipendula* (16%)
3	122	67	*Carex* (64%)
4	65	60	*Carex* (54%), *Geum* (16%)
5	252	65	*Carex* (46%), *Poa* (12%), *Equisetum* (10%)
6	191	75	*Carex* (56%), *Festuca* (19%)
7	108	56	*Carex* (49%), *Galium* (8%)
8	70	64	*Carex* (53%), *Geum* (14%), *Festuca* (11%)
9	35	73	*Carex* (53%), Phragmites (14%), *Urtica* (13%)
10	262	57	*Carex* (57%), *Viola* (15%), *Thelypteris* (15%)
11	177	51	*Carex* (42%), *Viola* (9%), *Geum* (8%)
12	105	50	*Carex* (50%), *Filipendula* (17%)
13	48	43	*Carex* (36%), *Geum* (23%), *Viola* (16%)
14	254	49	*Carex* (49%), *Potentilla* (29%)
15	160	53	*Carex* (47%), *Ranunculus* (15%), *Potentilla* (13%)
16	90	34	*Carex* (33%), *Geum* (22%), *Filipendula* (13%)
17	38	37	*Carex* (37%), *Geum* (17%), *Filipendula* (15%)

sediment in 1% Na_2CO_3 solution over a 5 hour period with sub-samples taken at 3, 4 and 5 hours and neutralized as adapted by Conley & Schelske (2001). The extract was analysed for DSi and plotted against time and the y-intercept was considered to be the ASi content to correct for simultaneous dissolution of (silicate) minerals.

2.3 Porewater DSi concentrations

Porewaters were sampled in the upper 10 cm using Eijkelkamp polymerous rhizons (diameter 2.5 × 1.4 mm, 10 cm length) in June 2007. Porewater was sampled at random with 3 rhizons at every site, and the samples were pooled at each site. The pooled sample was analyzed for DSi concentration on an IRIS® ICP (Inductively Coupled Plasma Spectrophotometer, Thermo®).

2.4 Vegetation

Vegetation composition at all sampling sites was determined at four randomly chosen plots using a cover pin frame (75 points/frame) in June 2006 (Kent & Coker 1995). The combined cover of grasses (*Poaceae*) and sedges (*Cyperaceae*) was expressed as a percentage of total individuals counted, belonging to either of both plant families. Dominance was preferred over number of individuals, as it was assumed to better reflect the overall vegetation composition on a longer time scale, rather than current absolute number of individuals counted. A summary of the dominant plant genera (all species with 10% or more coverage) at every site is provided in Table 1, as well as the grass and sedge dominance.

2.5 Statistics

Extrapolation of ASi, DSi and vegetation structure over the study site was accomplished using the kriging software FIELDS (Reinhard *et al.* 2009) in the statistical software packet R (R Development Core Team 2008). All other statistical tests were performed using the statistical software package SPSS® 14.0. (SPSS Inc., Chicago, Illinois, USA)

← **Table. 1** Dominant vegetation (all genera with higher than 10% dominance in the plot), distance from the river and grass and sedge dominance (P+C dominance, as percentage of the total number of vegetation), summarized for all the sampling spots, as in Fig. 1 and 4.

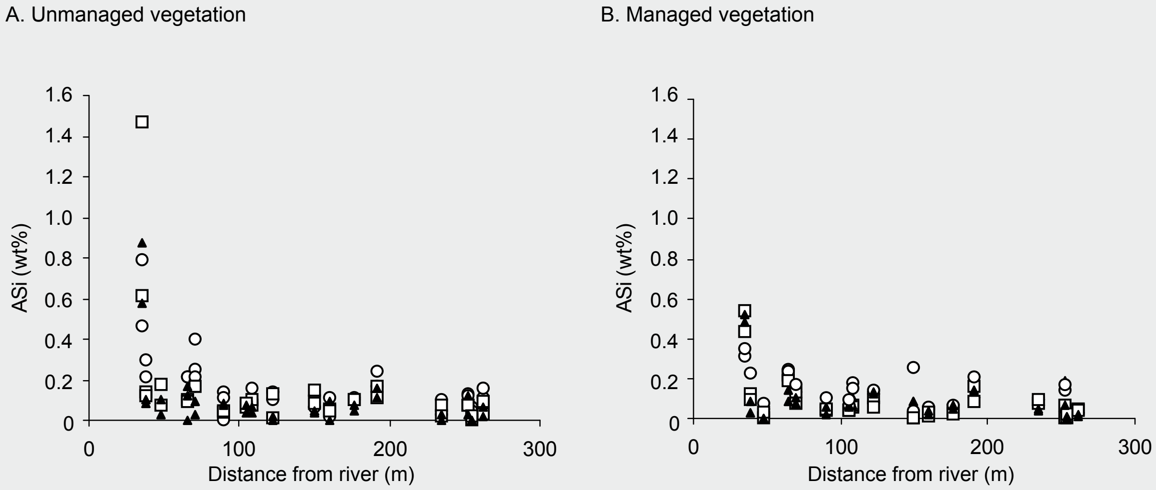
3. RESULTS

3.1 ASi concentrations

The highest ASi concentrations were observed at sites closest to the Biebrza River and, in general, in the top 10 cm of the soil profile (Fig. 3). However, at sites near the river the highest ASi concentrations were observed in deeper layers. The areal distribution of ASi in the upper 10 cm of sediment extrapolated over the study area also shows higher ASi concentrations closer to the river (Fig. 4B). General depth related trends in ASi concentration were investigated using paired two-tailed Wilcoxon signed rank tests (corrected for multiple testing), comparing depth sections within the 34 cores taken at sites with unmanaged vegetation and managed vegetation. All depth layers differed significantly for ASi concentration in the unmanaged vegetation (0-10 cm (L1) > 10-20 cm (L2) > 20-30cm (L3); L1, L2 $p < 0.002$; L1, L3 $p < 0.0001$; L2 , L3 $p < 0.005$). Paired Wilcoxon signed rank tests for sites in the managed vegetation indicated that the two deeper layers were not significantly different (0-10 cm (L1) > 10-20 cm (L2) = 20-30 cm (L3); L1, L2 $p < 0.0004$; L1, L3 $p < 0.002$; L2, L3 $p = 0.84$). These tests show that the surface layer is significantly enriched in ASi compared to deeper layers.

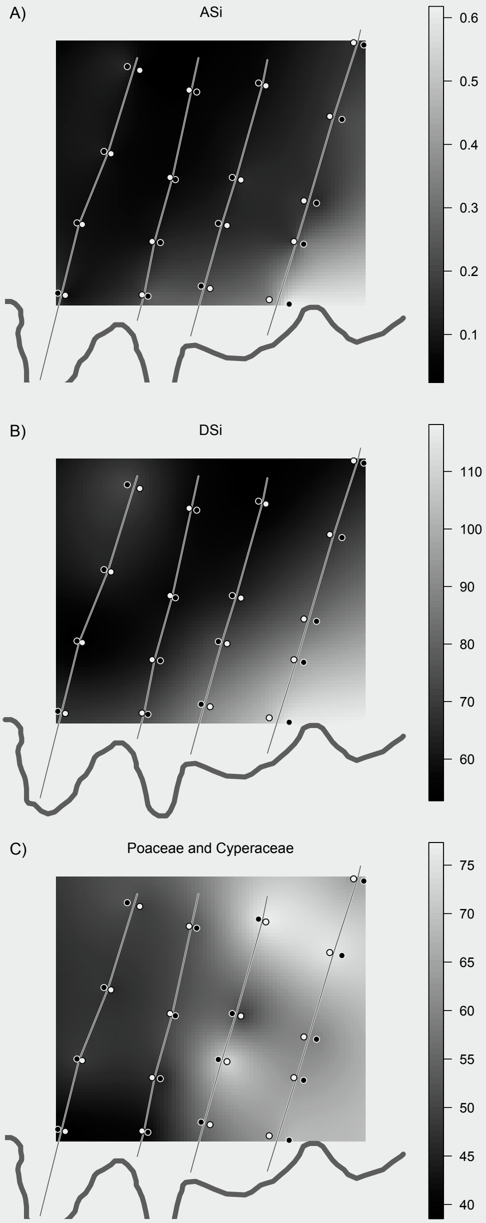
3.2 Effect of vegetation and distance from the river

The relationship between vegetation composition and proximity to the Biebrza River on ASi concentration was analysed for the upper 10 cm of soil. A significant logarithmic decrease of ASi concentrations in the top 10 cm of sediment with increasing distance perpendicular to the river was found both at managed ($R^2 = 0.29$; $F_{1,32} = 13.36$; $p < 0.001$) and unmanaged vegetation sites ($R^2 = 0.46$; $F_{1,32} = 27.48$; $p < 0.001$) (Fig. 5A). The relationship showing decreasing ASi concentrations with increasing distance from the river is also clear in Fig. 4B, albeit that the influence of vegetation partly masks the effect. However, there was no clear effect of combined grass and sedge dominance (or grass and sedge dominance separately, data not shown) on observed ASi concentrations in the upper 10 cm (Fig. 5B). Yet, accounting for the logarithmic river distance effect on ASi concentrations and "correcting" observed ASi concentration

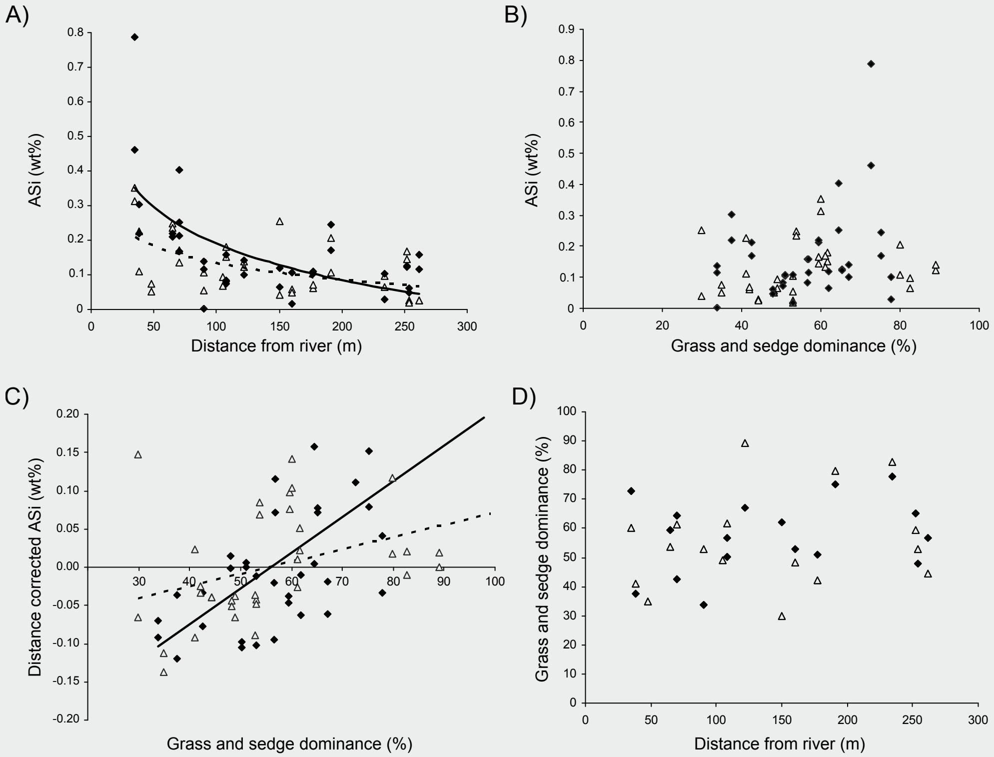


↑ **Fig. 3** ASi concentration (% ASi of sediment weight, wt%) in A) unmanaged and B) managed vegetation in relation to perpendicular distance from the river to the sampling site, in three different depth layers (○: 0-10cm; □: 10-20cm; ▲: 20-30cm).

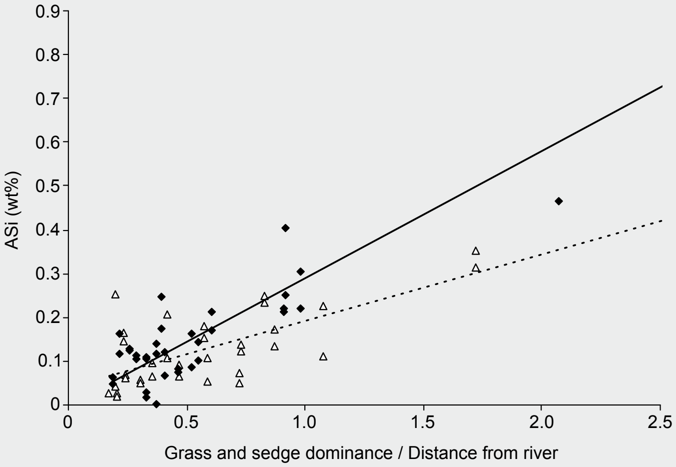
with the "expected" ASi concentration from the distance effect, an additional effect of vegetation was found (residual ASi = observed ASi – ASi expected from distance). A positive linear relationship between combined *Poaceae* and *Cyperaceae* dominance and residual distance corrected ASi is apparent in the top soil (Fig. 5C) in the unmanaged vegetation ($R^2 = 0.30$; $F_{1,32} = 14.00$; $p < 0.002$). A similar relationship was found in the managed vegetation (Fig. 5C), but the ASi concentration increase with dominance was less steep, and the relationship was only marginally significant ($R^2 = 0.13$; $F_{1,32} = 4.78$; $p < 0.04$). It is important to note that there was no significant linear relationship between the distance perpendicular to river and the combined grass and sedge dominance both at managed and unmanaged vegetation sites (Fig. 5D, Managed vegetation: $R^2 = 0.04$; $p > 0.46$, Unmanaged vegetation $R^2 = 0.08$; $p > 0.28$). Combining the grass and sedge dominance and the distance to the Biebrza River in one factor (by dividing the *Poaceae* and *Cyperaceae* dominance by perpendicular distance to the river), a significant linear relationship between



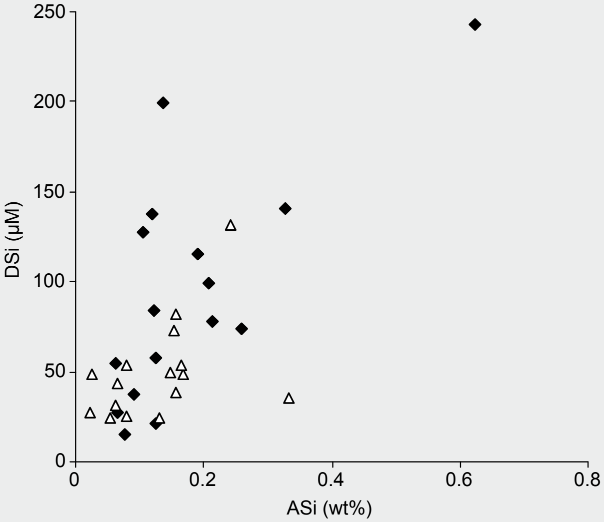
← **Fig. 4** Grass and sedge dominance (% of total number of plant individuals, panel A), ASi concentration (% ASi of sediment weight, wt%, panel B) and porewater DSi (µM, panel C) as observed over the Rogozyn site. Black dots are unmanaged vegetation sampling spots, open white dots managed vegetation. Red straight lines indicate the sampled gradients in distance from the river Biebrza.



↑ **Fig. 5** ASi patterns in both the unmanaged and managed vegetation. A) ASi in the top 10cm of soil vs. distance from the river. B) ASi in the top 10 cm of soil vs. grass and sedge dominance in vegetation. C) distance effect "corrected" ASi in top 10 cm of soil vs. grass and sedge dominance. D) distance from the river and grass and sedge dominance as independent variables. (Managed: △···, Unmanaged: ◆—)



↑ **Fig. 6** Relation between a factor combining vegetation and distance (vegetation dominance (%) divided by distance from the river (m)) and the distribution of ASi concentration in the top 10 cm of soil in the unmanaged and managed vegetation (Managed: △···, Unmanaged: ◆—).



↑ **Fig. 7** ASi in soil (averaged per two observations in the same site and the same management type) vs. observed porewater DSi concentrations. Observations are for both managed and unmanaged vegetation.

this new factor and observed ASi concentration was found, both in the managed and unmanaged vegetation. Combining vegetation and distance to the river we account for (R^2= 78%) ($F_{1,32}$ = 115.06; $p < .001$, R^2 for distance only was 46%) of the observed ASi variation at the unmanaged vegetation sites, and 51% of the variation at the managed vegetation sites ($F_{1,32}$ = 33.41; $p < 0.001$, R^2 for distance only was 29%) (Fig. 6). For both sites containing managed and unmanaged vegetation, the combined factor improves ASi variation accounted for by 60 and 70% compared to distance to the river alone. Both the distance from the river and the vegetation independently account for part of the ASi variation observed over the wetland. The combined effect of vegetation and distance is stronger at unmown sites. The combined influence of vegetation and distance from the river is also apparent in Figs. 4A and 4B. While the gradient associated with distance from the river is situated along a North-South axis, the grass and sedge dominance primarily increases from West to East. The result is highest ASi concentrations in the SE part and lowest concentrations in the NW part of the Upper Basin.

3.3 Porewater concentrations

For both managed and unmanaged vegetations, highest porewater concentrations were generally observed in the sites with highest ASi (Fig. 7). A significant positive linear relationship was actually observed between ASi concentrations (average for both observations in one site and one management type) in the top 10 cm and pooled porewater DSi concentrations at the same sites for the combined managed and unmanaged datasets (R^2 = 0.45; $F_{1,32}$ = 12.04; $p <0.001$). The association between soil ASi and porewater DSi is evident in Fig. 4B and 4C, where porewater DSi shows the same gradient as ASi concentrations.

4. DISCUSSION

We show here that the storage of ASi in riparian wetlands is significantly related to riverine connectivity and vegetation, as previously hypothesized by Struyf & Conley (2009). In this review, the authors showed that wetlands can profoundly impact the biogeochemical silica cycle through the activities of silica accumulating organisms such as diatoms, sponges, grasses and sedges, although few studies have documented this role of wetlands in silica cycling. Our data support this new understanding. In addition, we show that ASi storage in wetlands is highly variable on small spatial scales, and that recycling to DSi is directly affected by this variation. Our data suggest a link between vegetation (dominance of grasses and sedges) and ASi storage. Evidence linking vegetation to storage of ASi in soils has been available from other ecosystems. In forest soils, higher ASi concentrations in soil profiles occur in forests dominated by beech (relatively rich in ASi) as compared to pine, a species poor in Si (Bartoli & Souchier 1978, Bartoli 1983, Saccone *et al.* 2008). The largest accumulation of ASi in soils has been observed at the island of Réunion from the historical accumulation of phytoliths contained in bamboo, the most efficient Si accumulator among all higher plants (Meunier *et al.* 1999). Blecker *et al.* (2006) hypothesized that storage of ASi in grasslands also strongly depends on precipitation, with high precipitation and frequent soil water renewal stimulating export of DSi from the system, after dissolution of soil ASi.

4.1 The river distance proxy

In our study, river distance has been used as a proxy for hydrological characteristics and river connectivity. The proxy accounted for a significant fraction of the variation in ASi concentrations, independently from grass and sedge dominance. We hypothesize that the observed variation of ASi with distance from the river can be explained by a mechanism observed previously in tidal wetlands. During winter and spring, the Biebrza River floods the Rogozyn site. During these flooding events, the river imports suspended material into the floodplain, potentially co-importing ASi as both phytoliths and diatom frustules in suspended particulate matter. Within the wetland, stream velocities quickly drop, causing all but the lightest particles to be deposited near the river. In tidal marshes, it has been shown that most suspended matter is deposited close to the river or creek banks (Temmerman *et al.* 2003), and a similar process was observed in non-tidal floodplains (Asselman & Middelkoop 1995). Dense vegetation, as observed along the Biebrza, further stimulates rapid settling of particulate suspended matter. The highest import of allochtonous ASi is therefore expected near the river. Another possible mechanism for the enhanced ASi concentrations near the river, would be a tendency for more Si rich grasses and/or sedges to occur. The site richest in ASi (site 9) was characterized by a relatively important dominance of *Phragmites australis*, well known for its large content of ASi compared to most other grasses (Hodson *et al.* 2005, Struyf *et al.* 2005). Unfortunately, we have no data of ASi in aboveground biomass for the Rogozyn site.

Storage of ASi could also be directly related to groundwater hydrology. Frequent oxidation-reduction cycles have been proposed to stimulate the dissolution of ASi (Beckwith & Reeve 1963, Sommer *et al.* 2006) with the sorption of DSi into sesquioxides under oxygenated conditions and adsorbed DSi subsequently released after a return to reduced conditions with dissolution of iron oxides. We have no full hydrological characterization of the site, and cannot confirm if oxidation-reduction is more intense at sites farther from the river. However, at least one piezometer near to the river showed persistent reduced conditions. Permanent water-logging could also slow dissolution of ASi for long time-periods through the build-up of DSi concentrations in the soil water. At sites with more frequent flooding and flushing of porewater with a greater flow of water, enhanced dissolution would be expected. Finally, more permanent wet conditions could enhance the growth of diatoms and sponges enhancing the accumulation of ASi. It is clear that the link between ASi storage and recycling in wetlands and hydrology needs further study to quantify its impact on the small-scale variation observed.

4.2 Vegetation and management

Our results demonstrate that the pre-dominance of ASi rich vegetation can significantly stimulate ASi storage in wetland soils, as previously shown on larger spatial scales in different ecosystems (e.g. Bartoli 1983, Jensen *et al.* 1999). This would imply that *Poaceae* and *Cyperaceae* dominance, as frequently observed in wetlands, increases DSi availability in soils available to plants by building a recyclable pool of soil ASi. Increased DSi availability through dissolution of ASi could in turn enhance grasses' and sedges' competitiveness. DSi uptake by plants is well known to enhance their resistance to multiple stressors, and thus potentially their competitiveness (e.g. Epstein 1999, Massey *et al.* 2007). We hypothesize that ASi build-up as a result of biomass burial, and resulting high porewater DSi concentrations, might reinforce DSi availability and grasses' dominant position, resulting in a positive feedback loop. A continuous import of DSi from the river or from groundwater is a necessary condition for this hypothesis of surficial ASi build-up and should be fulfilled in a majority of riparian wetland sites, buffering groundwater and surface water fluxes between terrestrial and aquatic environments.

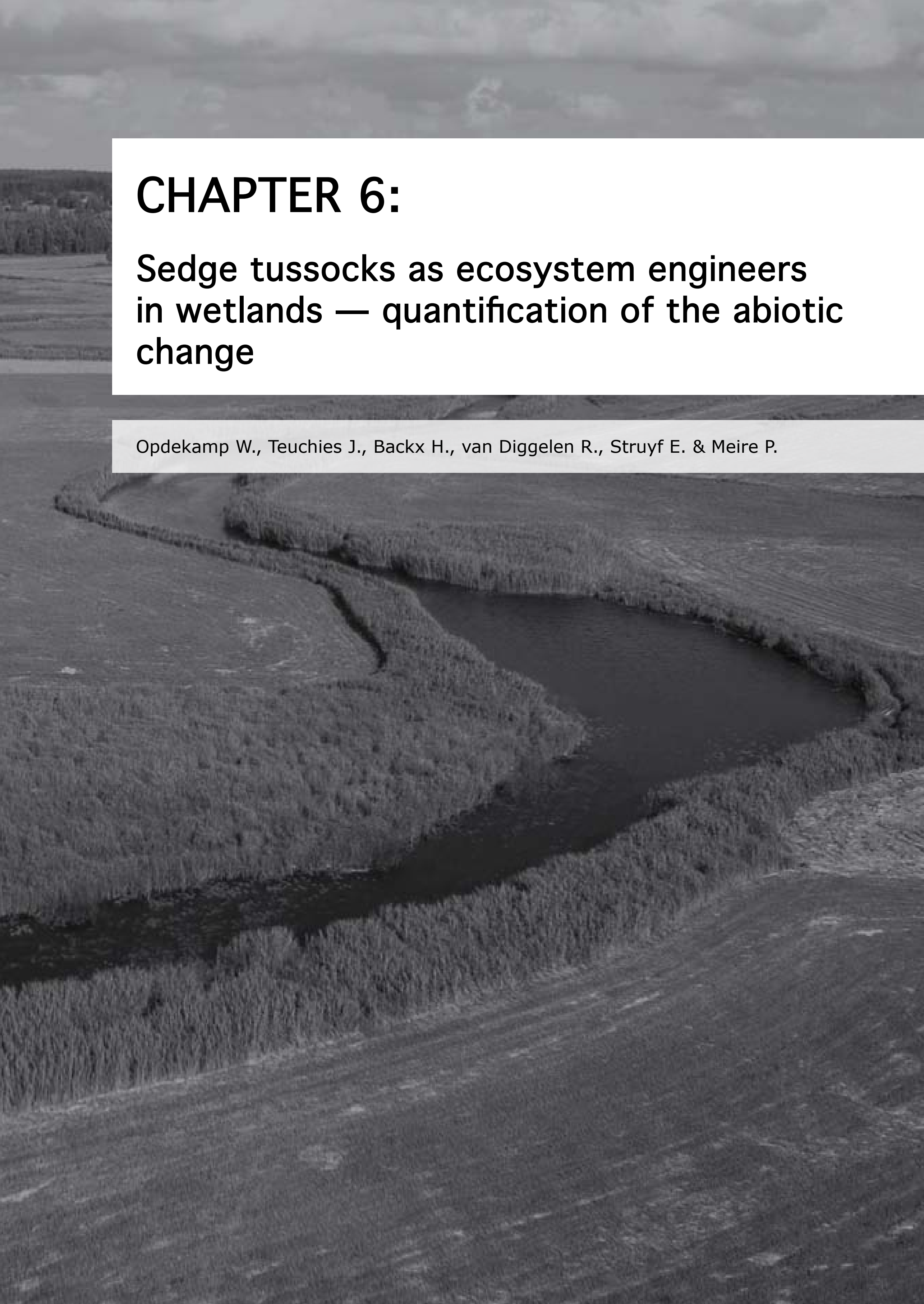
Riverine connectivity and vegetation accounted for more of the observed variation in ASi concentrations at sites not managed by mowing. This is coherent with increased trapping of allochtonous material in dense tussock vegetation and increased grass biomass burial in the absence of mowing. The ASi concentrations in our study site were low (<1% by weight), yet control of ASi on DSi concentrations was apparent. ASi is well-known for the control it exhibits on soil DSi concentrations (Farmer *et al.* 2005, Struyf *et al.* 2005), as it is more soluble than mineral silicates (Van Cappellen 2003). The depth gradient of preservation we observed also indicates dissolution of ASi with depth. The results imply that wetland management and its impact on ASi storage in wetlands, can consequently alter DSi available in the soil water. Export of water from the wetland to the aquatic environment mainly occurs as advective leakage to the river of soil water, especially during rain events, or through vertical leakage to the groundwater. As such, ASi dissolution probably controls export of DSi from the riparian habitat into the river system. In estuaries, wetlands are buffers in the silica cycle, exporting Si after recycling of buried ASi in times of Si-depletion (Struyf *et al.* 2006, Jacobs *et al.* 2008). Porewater DSi is the main source for the DSi export (Struyf *et al.* 2006). We suggest that a similar mechanism occurs in non-tidal wetlands.

4.3 Conclusions

An increasing number of studies now indicate that wetlands can form both sinks and sources for Si in river ecosystems (Struyf & Conley 2009). Our results show that relatively large variation in wetland ASi storage can occur on local, small spatial scales. We provide the first experimental evidence that distance from the river and vegetation can significantly influence ASi dynamics within wetlands over small spatial scales. The relative importance of local hydrology vs. allochtonous import of material could not be distinguished in this study, as distance from the river could be directly linked to both (although the presented data suggest that groundwater hydrology is rather uniform over the whole site). Future work should further constrain and identify these factors. Our understanding of the importance of wetlands to riverine Si biogeochemistry and the transport of Si along the land-ocean continuum is growing. To improve our understanding of both the sink and source functions, exchange studies in a variety of wetlands are a necessary prerequisite, as well as enhanced understanding of wetland Si dynamics. Our results indicate that vegetation and hydrology should be included as forcing factors in these studies. The importance of riverine Si transport in coastal productivity, where Si, N and P exhibit important controls on phytoplankton composition and productivity, certainly warrants a scientific effort that would enable our understanding of Si biogeochemistry to increase to the level of understanding achieved for N and P cycling.

ACKNOWLEDGMENTS

Fiona Franken was a great help during the vegetation mapping.



CHAPTER 6:

Sedge tussocks as ecosystem engineers in wetlands — quantification of the abiotic change

Opdekamp W., Teuchies J., Backx H., van Diggelen R., Struyf E. & Meire P.

ABSTRACT

In wetlands, plants and soil organisms are often exposed to harsh conditions. The physical stress they experience is strongly related to the hypoxic or anoxic status of waterlogged soils. We suggest that tussock growth in wetlands can be one of the possible structural changes to evade the detrimental effects caused by waterlogging.

In this study we investigated the feasibility of the Fibrous Tussock-sedge, *Carex appropinquata* A. Schumach, to escape from waterlogged conditions in a riverine wetland. In contrast with many previous publications on ecosystem engineering, we quantified the abiotic changes, i.e. redox potential and nutrient availability, induced by the engineering species. The nutrient and cation distribution was determined within tussocks, in the inter tussock space, as well as in the soil of an adjacent parcel where mowing prevented tussock formation.

Our results clearly show how tussock forming plants engineer their environment by creating their own aerobic environment above the waterlogged surroundings. The subsequent increase in redox potential and temperature within tussocks affected a cascade of biochemical processes. Increased mineralization rates resulted in an elevated nutrient availability, especially of nitrogen, the most limiting nutrient in this wetland. This resulted in a three-fold increase of the aboveground biomass production.

Furthermore, as many organisms are strictly oxygen-depended for their survival, these tussock sedges might act as ecosystem engineers through their effect on soil redox conditions. As such, they can have a profound effect on the fitness of species growing in their immediate surroundings. Yet, whether its effect on its surrounding species can be judged positive or negative, is clearly scale-dependent.

1. INTRODUCTION

All living organisms affect their surroundings. Yet, some species, known as ecosystem engineers, impose strong modifications which have a major impact on other organisms (Jones *et al.* 1994, Jones *et al.* 2010). Through their presence, physical ecosystem engineers control resource availability either directly or indirectly, while habitats are modified, maintained or created (Jones *et al.* 1997). Especially in harsh habitats, where environmental conditions constrain the development of well-established communities, small changes in certain physical conditions, induced by ecosystem engineers, can have a profound effect, both on species diversity and above-ground biomass distribution (Crain & Bertness 2005). Ecosystem engineering generally results in increased habitat heterogeneity, which can lead to higher species diversity (Jones *et al.* 1997). Despite the possible beneficial effects for some species, habitat transformation by a physical engineer can be harmful for others. Additionally, the consequences on biodiversity and ecosystem functioning depend to a large extent on the evaluation scale, e.g. local versus landscape scale (Jones *et al.* 1997).

Plants growing in cushions or tussocks are a well investigated example of habitat engineering. The plant structure grows out above its surroundings, changes the reigning abiotic conditions and thus creates a self-beneficial micro-environment. *Triglochin maritimum*, which forms raised rings above the salt marsh mud, creates a substrate with increased oxygen levels and a reduced salinity (Fogel *et al.* 2004). In boreal regions, higher temperatures have been observed within *Eriophorum vaginatum* tussocks, leading to a prolonged growing season and increased nutrient availability compared to inter-tussock soil (Chapin III *et al.* 1979). Tussock growth has been found to increase species richness in harsh environments like salt marshes (Fogel *et al.* 2004), subalpine regions (Yu & Ehrenfeld 2010), arctic regions (Chapin III *et al.* 1979), … as well as in more benign environments including freshwater marshes (Crain & Bertness 2005), grasslands (Perelman *et al.* 2003) and meadows (Peach & Zedler 2006).

In wetlands, plants and soil organisms are often exposed to harsh conditions (Mitsch & Gosselink 2000). The physical stress they experience is strongly related to the hypoxic or anoxic status of flooded and waterlogged soils (Colmer & Voesenek 2009). The resulting low oxygen concentrations prevent organisms from using aerobic metabolic pathways for respiration. Consequently, other ions than oxygen are progressively reduced, causing drops in redox potential (Mitsch & Gosselink 2000). These micro-organism induced electrochemical changes limit mineralization processes and lead to limited nutrient availability, especially of nitrogen (de Mars *et al.* 1996, Olde Venterink *et al.* 2002b). Reduced conditions can also cause increased levels of reduced compounds and available metals, even reaching phytotoxic levels (Mainiero & Kazda 2005). Therefore, many wetland plants possess specific mechanisms to deal with soil anoxia. These include metabolic adaptations as well as morphological escape mechanisms. The latter are mainly based on the development of aerenchym (Vartapetian & Jackson 1997). Some sedges can maintain aerobic root respiration via internal oxygen transport through the aerenchym (Mainiero & Kazda 2005). Additionally, the roots of some wetland plants release oxygen to the soil to create an aerobic rhizosphere, known as radial oxygen loss (Mainiero & Kazda 2005).

We hypothesize that tussock growth in wetlands is a structural change to evade the detrimental effects (e.g. anoxia, limited nutrient availability) caused by waterlogging. Tussock forming plants create a "personal" aerobic environment above their waterlogged surroundings. The subsequent increase in redox potential and temperature within tussocks affects a cascade of biochemical processes. We hypothesize that mineralization rate will be higher, resulting in an elevated nutrient availability. The resulting changes will be beneficial for the tussock forming plants themselves. We investigated the feasibility of the Fibrous Tussock-sedge, Carex appropinquata A. Schumach, to escape from waterlogged conditions in a riverine wetland. In contrast with many previous publications on ecosystem engineering, we quantified the abiotic changes, i.e. redox potential and nutrient availability, induced by the engineering species. The nutrient and cation distribution was determined within tussocks, in the inter tussock space as well as in the soil of an adjacent parcel where mowing prevented tussock formation. Finally, we critically evaluated the scale, from local to landscape, at which these abiotic changes affect plant species richness and the functioning of C. appropinquata as ecosystem engineer.

2. MATERIAL AND METHODS

2.1 Site descriptions

The Biebrza River valley, located in NE-Poland, harbours one of the best preserved wetlands in Central-Europe with few land reclamation and draining activities (Okruszko 1990). A detailed description of the valley can be found in Wassen *et al.* (1990). Our study area, located near Rogozyn in the Upper Basin (N53°42′16″, E23°26′30″), consists of (former) hay meadows on a sandy/ loamy peat soil (1 to 2 m deep, underlain by fluvial and eolian sand). The area is bordered by a sandy moraine in the north and the river Biebrza in the south, which creates a hydrological gradient from the moraine to the river. The water table fluctuates between -30 and +10 cm throughout the season with shallow flooding during late winter-early spring (Struyf *et al.* 2009). Until the late eighties to early nineties all parcels were subjected to an annual mowing regime (end of June-July). However, annual mowing has been abandoned between 1991 and 1995 (pers. comm. local farmers) in three parcels, which led to secondary succession and the development of sedge tussocks (up to 30cm high). In this study we selected two sites in adjacent parcels differing in management regime (continued mowing regime vs unmanaged).

In the nature reserve Damse Stadswallen near Damme, Belgium (N51°15′5″, E3°16′32″), floating rafts developed in the moats of the former Spanish fortifications (17th century). Present vegetation on this rafts is dominated by well-developed tussocks of the sedge *Carex paniculata* L.

2.2 Tussock analysis

At the Polish site, we selected eight well-developed tussocks of the sedge *C. appropinquata* A. Schumach. in the unmanaged parcel. Zero soil depth was arbitrarily defined as the mean value of the space in between tussocks (here-after called inter-tussock). Soil depths were either positive (up into the tussock) or negative (down towards mineral layer). We calculated the height for each tussock as the average elevation of the highest point of the tussock relative to four points evenly spaced around the base of the tussock. Each tussock was cut at zero soil depth using a serrated knife and divided into 10 cm high slices starting from zero soil depth upwards. For each slice the surface of the lower and upper section was determined in order to calculate its volume using the formula for a truncated cone. One soil core (Ø: 15.2 cm, H: 20 cm) was taken underneath every removed tussock. Additionally, eight 'inter-tussock' cores were taken in the unmanaged parcel. In the mown parcel 16 soil cores with the same dimensions (eight with *C. appropinquata* shoots present, eight without) were taken and separated into 10 cm high slices (Fig. 1A). All vegetation on top of a tussock or core was removed and sorted into living monocotyledons (mono), living moss (moss), living dicotyledons and equisetales (di_e) and dead material (dead), dried for 48h at 70°C and weighed.

After determining their fresh weight, each slice was divided into several parts. The first was dried at 70°C for 48h and was used to determine organic and moisture content. The second was used to determine concentrations of extractable nutrients and cations. Roots and belowground stem parts were sorted from the third part, washed gently to remove adhering soil particles, dried at 70 °C for 48 h and weighed. Roots and belowground stem parts were pooled and are reported as belowground biomass. Aboveground biomass on tussocks or soil cores was harvested and sorted into living monocotyledons, dicotyledons, mosses and dead biomass. Samples were dried for 48h at 70°C, weighed and expressed per m². All samples were taken in late June 2007.

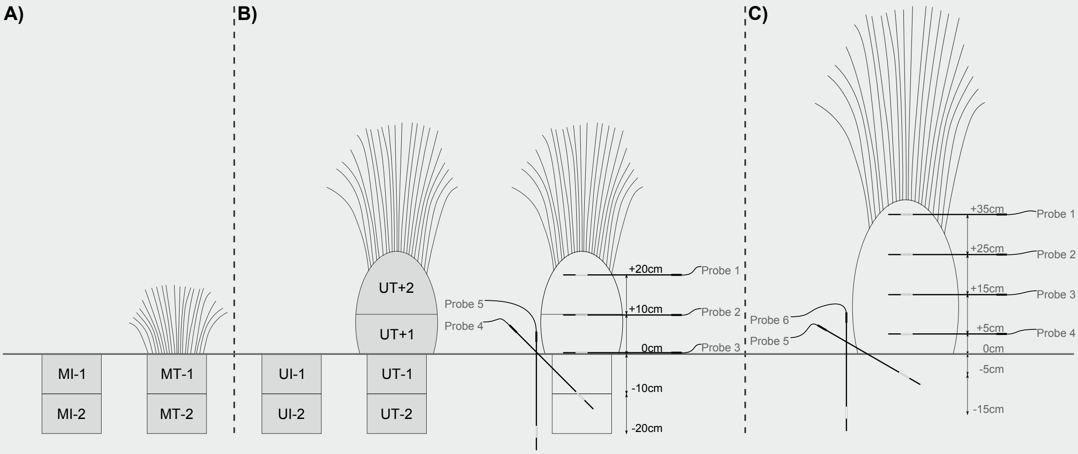
For the purpose of this paper, bulk soil is defined as the entire soil matrix, encompassing soil minerals, soil organic matter (SOM) and living belowground plant biomass, occurring within and beneath tussocks. Netto soil constitutes only of the soil minerals and SOM as the living belowground biomass (roots and rhizomes) has been removed. Roots and rhizomes are pooled and reported as belowground biomass.

2.3 Soil and vegetation analysis

Soil extractable N was determined after extraction of 40 g wet soil with 100 mL of a 1 M KCl solution (Houba *et al.* 1989), followed by colorimetric measurement of the $NO_{2/3}^-$ and NH_4^+ concentrations with a Skalar auto-analyser (Skalar, Breda, The Netherlands). Extractable PO_4^{3-} and K, Fe, Ca, Mg-ions were determined after an extraction of 20g wet soil with 100 mL of a 0.5 M ammonium acetate-EDTA (pH 4.65) solution (Cottenie *et al.* 1989). PO_3^{4-} concentrations were measured colorimetrically using a Skalar auto-analyser. K, Fe, Ca and

Mg-concentrations were determined using a Thermo Iris ICP (Thermo Fisher Scientific, Waltham, USA). pH-KCl was determined in a 1:2.5 (w:v) solution (Houba *et al.* 1989). Soil organic matter content was estimated through loss on ignition (Houba *et al.* 1989) by determining the weight ratio of oven dried (105 °C) versus incinerated soil (6 h at 550 °C).

Total N, P and K concentrations of bulk soil, belowground and aboveground plant biomass were determined following an acid digestion (Walinga *et al.* 1989). Concentrations were determined on a colorimetric segmented flow analyzer (Skalar, Breda, The Netherlands) for N and P and on a Thermo Iris ICP (Thermo Fisher Scientific, Waltham, USA) for K.



↑ **Fig. 1** Sampling design for the determination of soil characteristics and measurement of Eh-values. Shaded areas indicate the tussock and inter-tussock parts sampled in the managed (A) and unmanaged site (B). The heights at which redox probes were inserted in and next to tussocks of C. appropinquata and C. paniculata are respectively shown in B) and C).

2.4 Redox

At the Polish site, continuous and automated redox potential measurements were conducted using measuring probes and a silver (Ag/AgCl) reference electrode connected to a data logger (Hypnos III, see Vorenhout *et al.* (2004) for technical details of Hypnos). The measuring probes consisted of a 30 cm epoxy rod (Ø 8 mm) with a 5 mm² Pt measuring sensor near the tip. In 2010, 16 probes divided over three *C. appropinquata* tussocks adjacent to the harvested tussocks in Poland, were inserted vertically near the tussocks, under the tussocks and horizontally in middle of the tussocks, on different heights (Fig. 1B). Temperature and redox potential was logged every 15 min over a time period of two weeks. Standardized redox potential values (E_h) were calculated as measured values (E_m) added to the potential of the silver reference probe (E_{ref} = 214 mV at 15 °C).

At the Belgian site, long term variation in redox potential was investigated in tussocks of a sister species, *C. paniculata*, with the same type of reference electrode and logger (Hypnos III). Here, the measuring probes consisted of a 30 cm epoxy rod (Ø 8 mm) with a 750 mm² stainless steel measuring sensor near the tip. Forty-eight measuring probes, equally divided over eight *C. paniculata* tussocks, were comparably inserted vertically near a tussock, underneath a tussock and horizontally in the middle of the tussock on 5, 15, 25 cm above ground level (Fig. 1C). Redox potential was logged every 15 min between April 20th and September 1st 2010. Technical errors caused a data gap from July 1st to August 10th.

2.5 Data analysis

A linear mixed-effect model with post-hoc comparison was used to test differences in soil parameters among tussock compartments. Data were square-root, log or arcsin-transformed when necessary to improve homogeneity of variances. Means and standard errors shown in Fig. 3, 4 and 5 were calculated on transformed data and then back transformed to aid visual representation. Analyses were performed with R 2.10.1 (R Development Core Team 2009). Linear mixed-effect models were run using the nlme package (Pinheiro *et al.* 2010) with Tukey's pairwise comparison following Hothorn *et al.* (2008).

3. RESULTS

3.1 Redox potential

At the Belgian site, E_h-values were highest in the upper part of the tussock and decreased towards the lower parts (Fig. 2A). In the upper tussock layers (both upper probes), E_h-values were higher than 350 mV with only little variation throughout the season (Fig. 2A). Values measured at an intermediate height (probe 3) varied around 330 mV throughout the season while values in the lowest part of the tussock were highly variable, ranging between -150 and +150mV. Values measured underneath and in between tussocks were markedly lower. After a sudden drop in early May, they slowly declined to stabilize around -200mV. Despite the short measuring period of two weeks at the Polish site, E_h-values showed a similar pattern with a clear distinction between values measured within tussocks and those measured underneath or in between (Fig. 2B). Temperature values in the upper soil layers were generally higher, but also more variable than in the lower layers or deep in between tussocks (Fig. 2C).

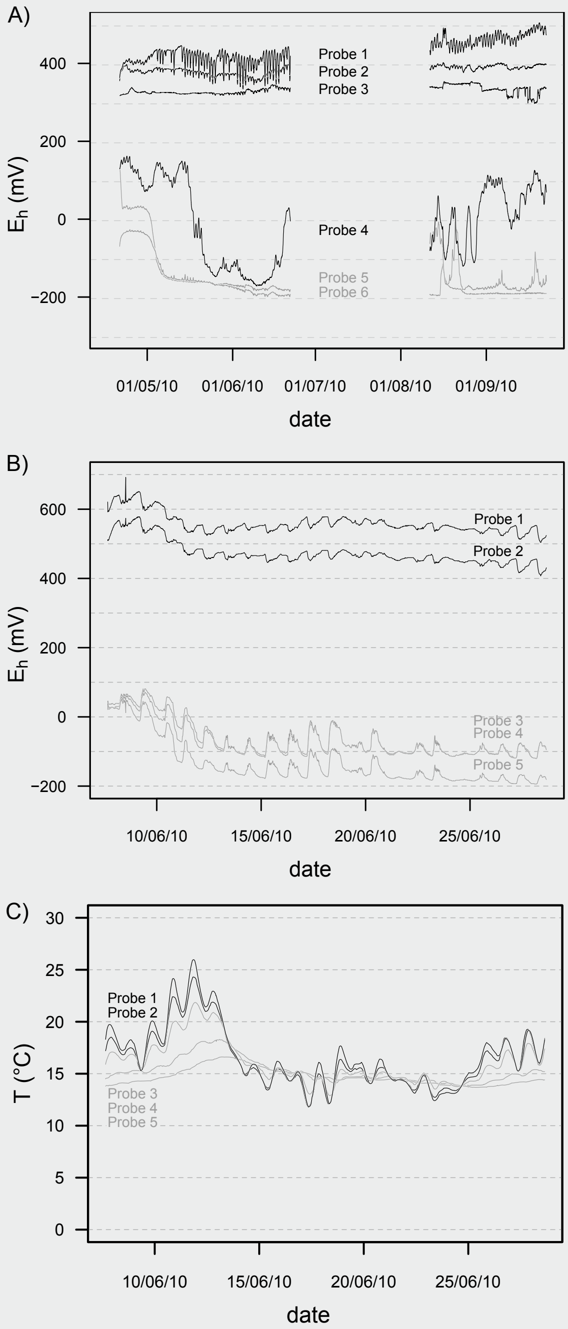
3.2 Aboveground Vegetation

Aboveground biomass was highest on top of well-developed tussocks (2800 g m^{-2}) and monocotyledons (mainly *C. appropinquata*) contributed more than 98% (Fig. 3). However, aboveground biomass production in tussock-forming patches in the managed parcel was significantly less (912 g m^{-2}). Here, monocotyledons only accounted for 75%. The N:P ratio of monocotyledons (mainly *C. appropinquata*) in patches of tussock-forming plants in the mown site was lower than the N:P ratio of monocotyledons on top of well-developed tussocks in the abandoned site (respectively 8.09 and 9.36; Welch Two Sample t-test, t = 2.6157, df = 10.199, $p = 0.025$).

3.3 Soil properties and nutrient concentrations

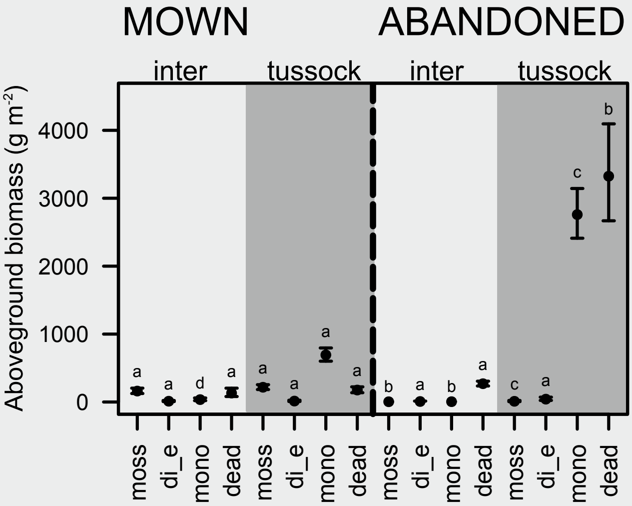
In the mown parcel, soil bulk density was highest in the deepest layers (210 mg cm^{-3}), but did not differ significantly between patches with and without tussock-forming vegetation. In the upper layer it is lower when tussock-forming vegetation is present. In the abandoned parcel, there were no big differences in soil bulk density among the different soil compartments in layer -1 and -2. However, the two upper tussock layers showed a significantly lower bulk density (120 mg cm^{-3}, Fig. 4A). Compared to soil bulk density, belowground plant biomass showed an opposite pattern. In the deepest layer of well-developed tussocks, belowground biomass was only slightly higher than in-between tussocks. In the upper tussock layer, belowground biomass was highest and accounted for almost half of the bulk density (Fig. 4A, B). Both moisture and organic matter content were high (both around 80%) and showed little variation. Only in the upper tussock layer they were respectively considerably lower and higher than in the other compartments (Fig. 4C, D). pH ranged from 5 to 6 and showed relatively little variation among the different compartments. Yet, in well-developed tussocks pH gradually increased from the deepest to the higher layers but showed a clear drop in the upper tussock layer (Fig. 4E). Total soil nitrogen content had an almost identical pattern as soil bulk density with highest values (6 mg cm^{-3}) in the deepest layer in the managed parcel and lowest values (1.7 mg cm^{-3}) in the upper part of well-developed tussocks (Fig. 4F). In contrast with nitrogen, the pattern of total soil phosphorus content did not coincide with that of soil bulk density. Except for patches with tussock-forming sedges in the managed parcel, total soil phosphorus content was higher in layer -1 than layer -2. Lowest total soil phosphorus content was observed in the upper layers of well-developed tussocks (Fig. 4G). In contrast to nitrogen and phosphorus, the total soil potassium content did not show a distinct pattern with clear differences between compartments in layer -1 and -2. However, total potassium was significantly higher in the upper tussock layer than in the other compartments (Fig. 4H).

In well-developed tussocks in the unmanaged site, $NO_{2/3}^-$ concentrations showed a clear vertical profile as they increased from the upper layer towards the lower layers. In the mown site, no distinct vertical profile could be observed within patches of tussock-forming vegetation. In between tussocks, $NO_{2/3}^-$ concentrations were higher, although non-significant, in the upper compared to the lower soil layer, both in the mown and unmanaged site (Fig. 5A). Just as for $NO_{2/3}^-$, concentrations of NH_4^+, PO_4^{3-} and K^+ followed a vertical profile within well-developed tussocks in the unmanaged parcel. Yet, in contrast with $NO_{2/3}^-$, the highest concentrations were found in the upper layers and decreased towards the lower layers. In between well-developed tussocks and in the mown site concentrations of these nutrients were (significantly) higher in the upper



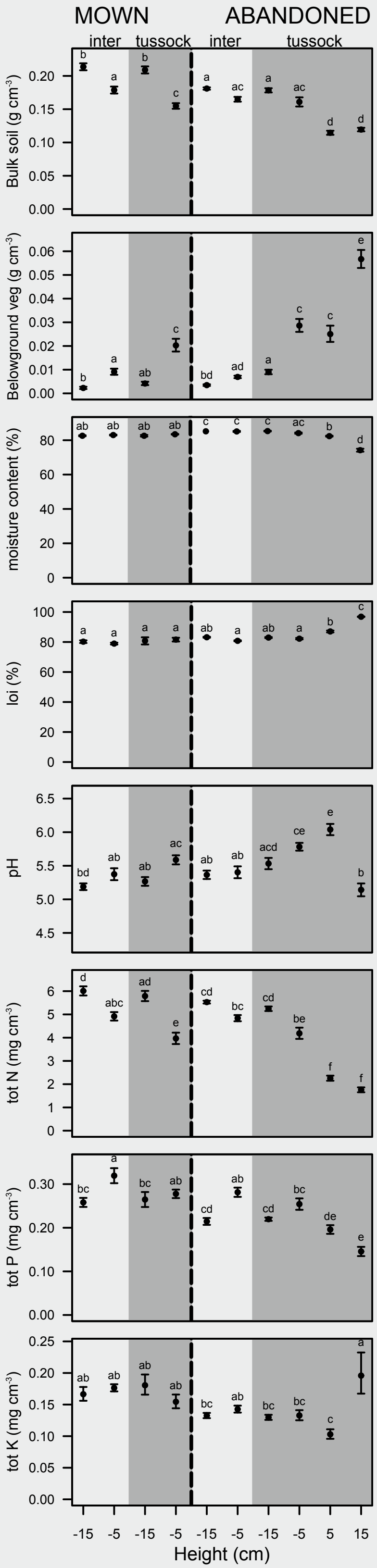
↑ **Fig. 2** Eh-values measured in tussocks of (A) C. paniculata and (B) C. appropinquata. Temperature values for C. appropinquata given in (C) are from the same tussock as (B). For clarity reasons, the results shown are limited to only one tussock. The heights at which probes are inserted within a tussock are shown in Fig. 1B and 1C.

soil layer (Fig. 5B, C & D). Extractable $Fe^{2+/3+}$ concentrations in the upper layer of well-developed tussocks were almost reduced to zero, but gradually increased towards lower layers. In between well-developed tussocks, $Fe^{2+/3+}$ concentrations in the upper and lower soil layer did not differ. In the mown site, concentrations in the upper soil layer were only slightly, but not significantly, higher than in the lower soil layer (Fig. 5E). Ca^{2+} concentrations showed little variation and ranged from 12mg cm^{-3} to 17mg cm^{-3} with slightly higher concentrations within well-developed tussocks. Yet, Ca^{2+} concentrations in the upper tussock layer were remarkably lower (Fig. 5F). Mg^{2+} concentrations were lower in deeper soil layers. Within well-developed tussocks a gradual increase in Mg^{2+} concentrations was observed with a clear drop in the upper layer (Fig. 5G).

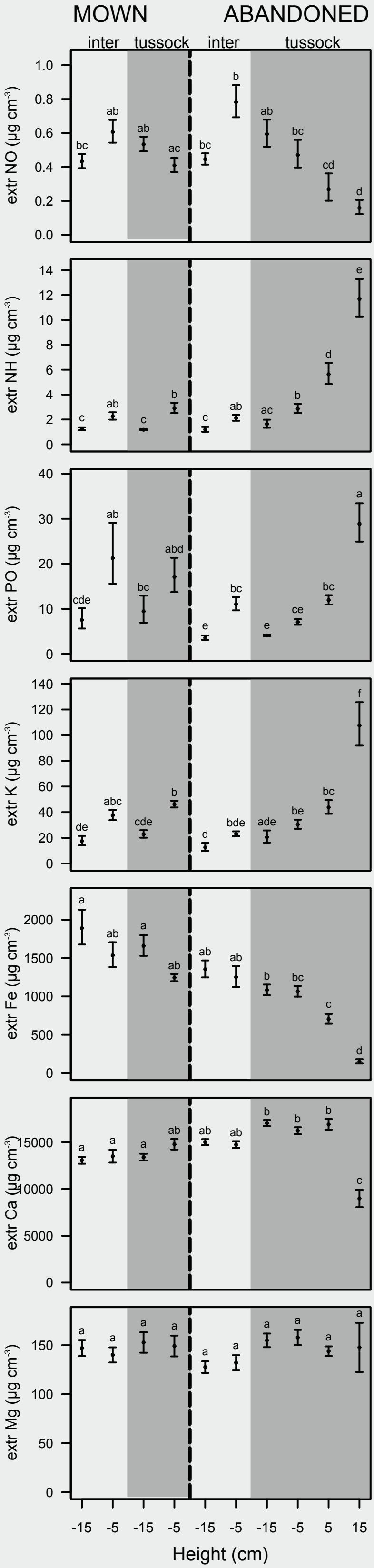


↑ **Fig. 3** Aboveground biomass in the mown and abandoned site, separated in living moss (moss), dicotyledons and equisetales (di_e), monocotyledons (mono) and dead material (dead). Significant pairwise differences within one type are indicated by different letters above the error flags ($p < 0.05$).

→ **Fig. 4** Soil parameters, total and extractable nutrient concentrations in the sampled tussock and inter-tussock compartments. Significant pairwise differences are indicated by different letters above the error flags ($p < 0.05$). Data were square-root, log or arcsin-transformed when necessary to improve homogeneity of variances. Means and standard errors shown were calculated on transformed data and back transformed to aid visual representation. For location of the sampled compartments, see Fig. 1.



→ **Fig. 5** Concentrations of extractable nutrients in the sampled tussock and inter-tussock compartments. Significant pairwise differences are indicated by different letters above the error flags ($p < 0.05$). Data were square-root, log or arcsin- transformed when necessary to improve homogeneity of variances. Means and standard errors shown were calculated on transformed data and back transformed to aid visual representation. For location of the sampled compartments, see Fig. 1.



4. DISCUSSION

4.1 Structural change and benefits

Our study indicates that escaping reduced conditions could be a prime driver for tussock formation, which thus forms a stress avoidance strategy to evade the detrimental affects of soil waterlogging (van de Koppel & Crain 2006). By elevating their rooting substrate, tussock-forming plants create a self-beneficial environment. We here show that tussocks experience more favourable redox potentials. E_h-values measured underneath and in-between tussocks are typical for waterlogged soils where all oxygen has been consumed (DeLaune & Reddy 2005). Wetland plants dispose of a wide range of traits to grow under oxygen depleted conditions (Vartapetian & Jackson 1997, Perata *et al.* 2011). The principal adaptation in different *Carex* species is aerenchym development to supply roots with oxygen (Visser & Bögemann 2003, Banach *et al.* 2009). Nevertheless, even well-adapted plants might experience severe stress at strongly reduced conditions (Brix & Sorrell 1996, Pezeshki 2001). Soil E_h-values in this study ranged from -150 to 0 mV, making iron and sulphate the main electron acceptors in microbial respiratory cycles (de Mars & Wassen 1999, DeLaune & Reddy 2005). This might lead to internal root anoxia or accumulation of reduced components up to phytotoxic levels, which on its turn might result in reduced plant growth or even death (Brix & Sorrell 1996). High E_h-values (between 350 and 600 mV) in the upper tussock layers imply a sufficient oxygen supply to maintain aerobic respiration and decomposition. Redox potential and water table are often strongly correlated (Dwire *et al.* 2006, Niedermeier & Robinson 2007). This is confirmed by a shift from reduced to oxidized conditions in the lower tussock layers as the water table lowered during the growing season.

In boreal regions, tussocks have a more favourable thermal regime, receive more irradiance and hence experience a prolonged growing season compared to inter-tussock soil (Chapin III *et al.* 1979). Although our temperature data are limited, *C. appropinquata* tussocks probably also experience a more favourable thermal environment compared to inter-tussock areas. Finally, our Polish study site gets flooded shallowly during late winter-early spring. Consequently, inter-tussock areas and managed sites will suffer longer from flooded conditions than the tussock and the onset of the growing season will be later. The combined effect of the favourable thermal regime, the higher redox potential and the earlier onset of the growing season give the tussock forming plants a potential advantage to their neighbouring plants.

4.2 Nutrient availability

Differences in redox potential affect biogeochemical cycles (de Mars & Wassen 1999). In anaerobic soils, decomposition rates are slow, often leading to nutrient limitation (Mitsch & Gosselink 2000). Aerobic conditions in the tussocks potentially enhance nutrient cycling (Olde Venterink *et al.* 2002a), confirmed by the high concentrations of ammonia (NH_4^+), the first N-molecule produced during organic nitrogen decomposition. Despite a low bulk density and the lower concentrations of total nutrients, upper tussock layers showed higher concentrations of extractable ammonium, phosphorus and potassium than does the underlying soil. These nutrients are the most important that potentially limit above-ground biomass production in wetlands (Olde Venterink *et al.* 2001). N:P-ratios suggest that the aboveground biomass production of monocotyledons, both in the managed and unmanaged parcel is limited by nitrogen (Güsewell 2004). Yet, based on these N:P ratios, monocotyledons on top of well-developed tussocks seem to be less limited by nitrogen than monocotyledons in the managed parcel. Consequently, by creating tussocks, these plants enhance mineralization processes which finally resulted in a three-fold increase of the aboveground biomass production. Likewise, Chapin III *et al.* (1979) found *Eriophorum vaginatum* leaf biomass per unit area measured in the centre of tussocks to be five-fold greater than the total vascular leaf biomass averaged over the whole site.

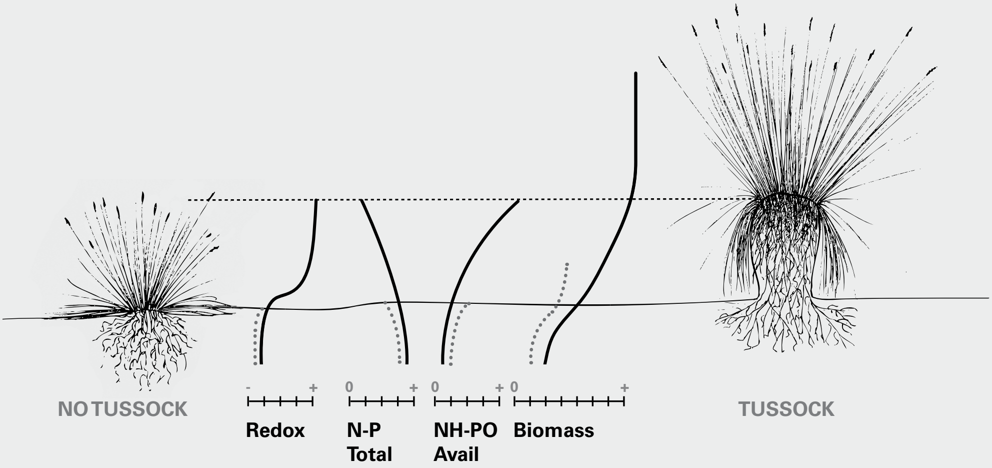
Inevitably, some mobile nutrients will be lost, either through leaching or through litter burial. To avoid deficiencies, tussock forming sedges need to be able to replenish these. High belowground plant biomass within and just underneath tussocks, mainly consisting of roots, indicates the ability of the tussock sedges to extract nutrients from the surrounding soil. The effectiveness of the roots to pump up nutrients into the tussock is further confirmed by the high K concentrations found in the upper tussock layers (Chapin III *et al.* 1979). Furthermore, as nutrient resorption from fine roots might be limited (Nambiar 1987, Dubach & Russelle 1994), this dense root network within and just below tussocks might partly take up the nutrients lost after root death.

To conclude we can state that the structural change, i.e. tussock formation, induced by these sedges affected a cascade of biochemical processes which finally lead to increased aboveground biomass production. As such, these tussock forming sedges can be considered as ecosystem engineers.

4.3 Sedge tussocks as ecosystem engineers

Plant communities are structured by a complex combination of facilitative and competitive interactions among species (Callaway & Walker 1997). In general, facilitative interactions are more important in harsh environments (Bertness & Hacker 1994). Facilitation by tussock forming plant species through the provision of a suitable habitat for others has been observed frequently (Chapin III *et al.* 1979, Levine 2000, Perelman *et al.* 2003, Fogel *et al.* 2004, Crain & Bertness 2005, Peach & Zedler 2006, Ervin 2007, Yu *et al.* 2010). Likewise, *C. appropinquata* physically modifies its abiotic environment and creates a new structural state through tussock formation (Tomaszewska 1993). This structural change modifies resource availability as the creation of an aerobic habitat above the waterlogged soil results in an altered biogeochemical cycle and subsequently higher nutrient availability within the tussocks. This structural and abiotic change will affect other species and inevitably results in a new biotic state (Jones *et al.* 2010). However, these changes are only positive for some species and can even be negative for others (Jones *et al.* 1997).

Generally, tussock growth increases surface area, which results in many micro-habitats with a broad range of hydrological and other environmental conditions, thought to provide a suitable habitat for more species than a more homogeneous environment (Vivian-Smith 1997, Silvertown *et al.* 1999, Peach & Zedler 2006). However, the environment created by tussocks is often also inhospitable for many plants. The space in between tussocks is characterized by reduced light penetration, increased litter deposition or intensified herbivory (Crain & Bertness 2005), while competition for light and other recources might be severe on top of a tussock (Levine 2000). In our study, most living aboveground biomass was found on top of the tussocks as an inhospitable inter-tussock space with low redox potential, low light (Opdekamp *et al.* 2012) and nutrient availability was created. Concordant with Crain & Bertness (2005), we conclude that a higher species richness related to tussock formation is driven here by the negative impact of tussocks on its surroundings. However, at this



↑ **Fig. 6** Conceptual figure summarizing different gradients, and their evolution following tussock development. Tussocks raise themselves above their waterlogged surroundings. Subsequently, Eh-values increase strongly, favouring aerobic decomposition processes. This leads to a strong increase of plant-available nutrients. Yet, total nutrient content is lower, due to the lower bulk density. Finally, this increased nutrient availability gets translated into higher aboveground biomass production. (Tussock: ——, No tussock: - - -)

local scale, there is no evidence that the presence of tussocks as ecosystem engineers results in either an increase or decrease of the total number of plant species at a larger scale (Jones *et al.* 1997).

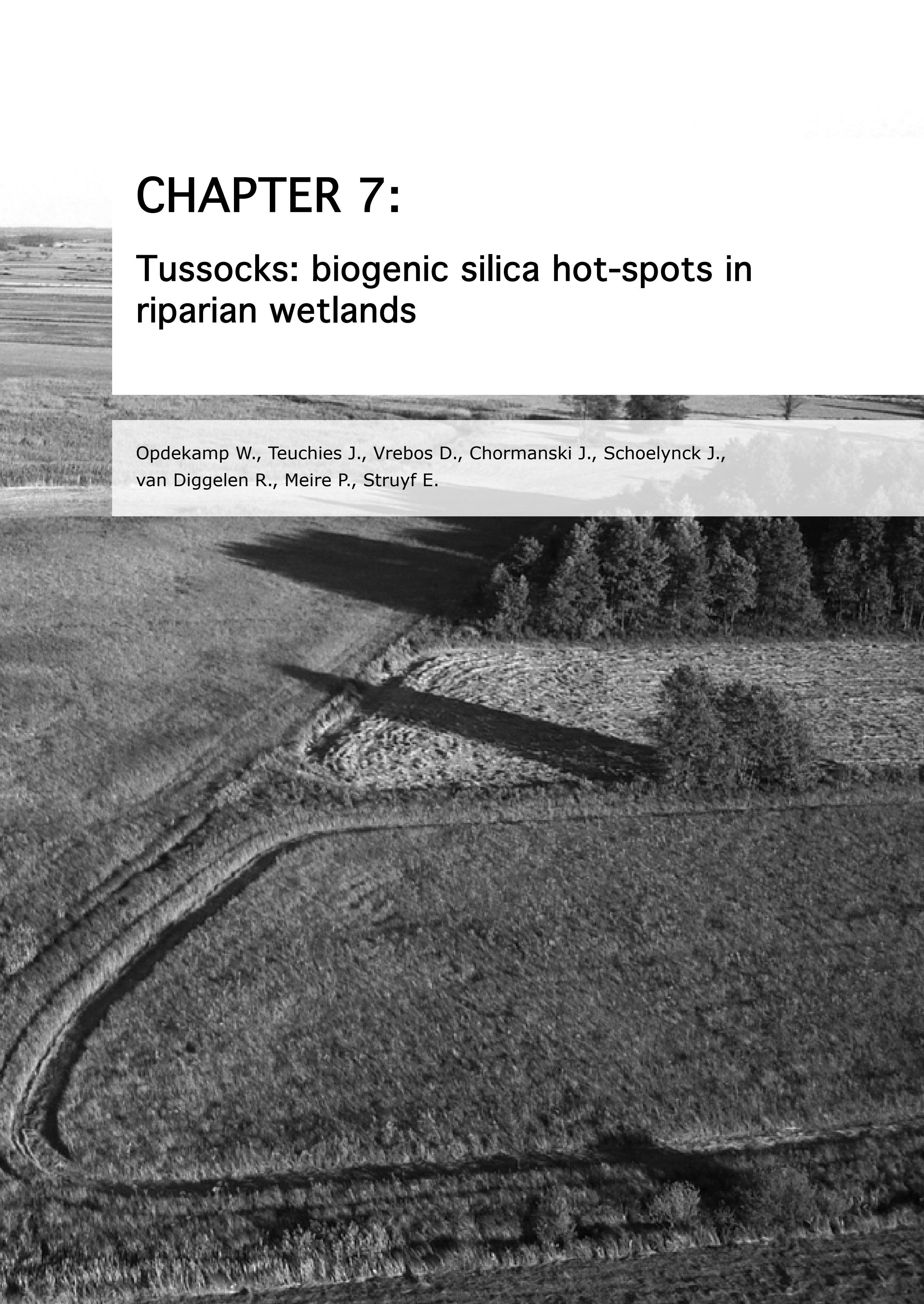
The balance between facilitation and competition by tall sedge tussocks in riparian meadows might depend on several factors. As discussed before, facilitation might become more important with increasing abiotic stress (Callaway & Walker 1997). Next, the effect of an engineering species on total species richness might depend on the evaluation scale (Jones *et al.* 1997). As tussock growth creates inter-tussock areas with very harsh conditions, other plant species are almost exclusively growing on the tussocks. Hence, on individual tussock scale, a positive effect of tussocks on plant species richness might be concluded. Yet, a continued mowing management with subsequent absence of tall tussocks reduces inter-specific competition resulting in higher species richness in anually mown meadows (Opdekamp *et al.* 2012). On the other hand, on an even larger scale in time and space, ecosystem engineering might have a positive effect on plant species richness. Tussock formation is a common stage in the succession from low productive meadow towards woodland. Even if this successional change results in a decrease in total species, some species might still profit. Especially species with a very complementary resource specialization might benefit from the occurrence of sedge tussocks (Herbert *et al.* 2004). A mosaic of habitats with and without tussocks in space (over a gradient in relation to the river) and time (over different successional stages) might thus increase species richness on the landscape level (Jones *et al.* 1997).

4.5 Conclusion

This study clearly shows how tussock growth forms a structural escape mechanism for plants growing in waterlogged conditions. By developing tussocks, they raise themselves above their waterlogged, anoxic surroundings. The subsequent rise in redox potential allows for aerobic decomposition processes to take place, leading to increased nutrient availability, especially of the nitrogen (the most limiting nutrient), within tussocks. This, on its turn, results in threefold higher aboveground biomass production. Hence, this structural change is beneficial for the tussock. These structural and abiotic changes are summarized in Fig. 6. By changing its environment, *C. appropinquata* also acts as an ecosystem engineer. Yet, whether its effect on its surrounding species can be judged positive or negative, is clearly scale-dependent.

ACKNOWLEDGEMENTS

We want to thank the Board of the Biebrza National Park and Natuurpunt for their permissions to work in their areas. Tomek Okruszko and the whole SGGW crew are acknowledged for the coordination of the joint measuring campaigns.



CHAPTER 7:

Tussocks: biogenic silica hot-spots in riparian wetlands

Opdekamp W., Teuchies J., Vrebos D., Chormanski J., Schoelynck J., van Diggelen R., Meire P., Struyf E.

ABSTRACT

The role of wetlands in Si cycling has remained largely unstudied. Yet, understanding the factors determining the size and extent of Si cycling in wetlands is important. They have been identified as important Si filters between terrestrial and aquatic ecosystems. Recently, five key factors have been highlighted as research focal points to fully understand the functioning of the ecosystem silica filter. One of these key factors is the magnitude and size of the Si reservoir in ecosystems, which strongly depends on the occurrence of organisms, specialized in biological Si processing.

Our study was aimed to test the hypothesis that tussocks, a common growth form of sedges in wetlands, can efficiently retain BSi. As such, tussock-forming graminoids could take advantage of an efficient Si processing mechanism, a nutrient that provides them with a competitive advantage over other plant species.

Our results show that tussock development causes a patch-like distribution of biogenic silica (BSi) stocks in wetlands. While in a managed wetland BSi is uniformly distributed over the surface layers, tussock development strongly interferes with this pattern. A mosaic of BSi richer inter-tussock soils and BSi poorer soils under tussocks develops, which results from the active uplift of Si by the tussock forming species into the tussock and the aboveground biomass.

More and more results show that wetlands play a key role in mediating Si fluxes between the aquatic and terrestrial environment. Our study shows that biogeochemistry and availability of Si changes in tussock-dominated wetlands compared to managed wetlands without tussocks. Tussocks will have an effect on the role of wetlands as silica hot-spots and biogenic Si sinks near rivers. This implies that future studies should focus on quantifying the effect of tussock development, and human management, on system scale BSi storage.

1. INTRODUCTION

Wetlands are well known hotspots for nitrogen and phosphorus cycling. Nevertheless, their role in Si cycling has remained largely unstudied, which is mainly attributable to the relatively recent recognition of the importance of terrestrial ecosystems (Conley 2002), including wetlands (Struyf *et al.* 2006, Struyf & Conley 2009), in controlling terrestrial Si fluxes. The annual fixation of dissolved Si (DSi) into terrestrial vegetation is more than one order of magnitude higher than the yearly export of DSi and suspended biogenic Si (BSi) from the terrestrial geo-biosphere to the coastal zone (Conley 2002). Understanding the factors determining the size and extent of Si cycling in ecosystems is important; Si-N-P ratios are essential in controlling coastal eutrophication and associated shifts in coastal phytoplankton communities (Cloern 2001). Furthermore, the silica-associated diatom carbon pump depends upon the annual input of terrestrial Si into the coastal zone and eventually the ocean (Tréguer *et al.* 1995, Laruelle *et al.* 2009).

Wetlands represent a link between the terrestrial and aquatic environment (Mitsch & Gosselink 2000). Lowland wetlands act as large filters between upstream ecosystems on the one hand and rivers and estuaries on the other hand. They are often characterized by fast plant growth and high biomass production, resulting in intense recycling and storage of N and P (Olde Venterink 2000). Struyf & Conley (2009) showed that wetland ecosystems may be as important for Si transport and processing as they are for other important biogeochemical cycles. Recently, five key factors have been highlighted as research focal points to fully understand the functioning of the ecosystem silica filter (Struyf & Conley 2012). One of these key factors is the magnitude and size of the ecosystem Si reservoir, which will be strongly dependent on the occurrence of organisms, specialized in biological Si processing.

Recent research emphasized the capacity of wetland and aquatic macrophyte communities to store BSi (Struyf *et al.* 2005, Schoelynck *et al.* 2010). Monocots, especially grasses (*Poaceae*) and sedges (*Cyperaceae*), often constitute a dominant part of wetland vegetation. They exert a strong influence on soil nutrient cycling (McLaren & Turkington 2010). In sedge meadows (Peach & Zedler 2006), floodplains (Wassen *et al.* 2002) and boreal wetlands (Chapin III *et al.* 1979), several sedge species form tussocks consisting of many tillers, sometimes reaching heights of up to 1 m, e.g. *Carex stricta* Lam. (Costello 1936). Tussocks provide relatively dry spots within a wetland, where more optimal growing conditions occur (Chapter 7, Crain & Bertness 2005). This tussock forming strategy is not limited to *Carex* sedges but is a common growth form observed in several graminoids, e.g. *Molinia caerulea* (L.) Moench (Taylor *et al.* 2001). Tussocks are often regarded as ecosystem engineers, creating suitable habitat for plant species in otherwise unfavourable habitat (Chapter 7, Peach & Zedler 2006).

Although grasses and sedges are well-known silica accumulators (Hodson *et al.* 2005) and nutrient cycling within tussocks has already been studied intensively (e.g. Chapin III *et al.* 1979, Jonasson & Chapin III 1991), Si has never been taken into account in these tussock studies. Yet, silica accumulation can provide plants with several competitive advantages, including the enhancement of growth and yield, an increased shoot rigidity and protection against physical stress, the promotion of photosynthesis, and a higher resistance to disease, herbivores, metal toxicity and salinity (Epstein 2001). High BSi content decreases the palatability of grass for herbivores, and higher levels of herbivory increase the investment in these defence structures (Massey *et al.* 2007). Furthermore, efficient use of Si by some plant species or varieties potentially influences their competitiveness in dynamic environments (Garbuzov *et al.* 2011). Struyf & Conley (2012) therefore hypothesized that graminoid tussocks could potentially alter Si availability by recycling BSi to DSi even before reaching the soil profile (as observed for other nutrients, e.g. Jonasson & Chapin III (1991)), as such making this key nutrient easily available to the tussock forming species.

Our study was aimed to test the hypothesis raised by Struyf & Conley (2012), which states that tussocks can efficiently retain BSi. As such, tussock-forming graminoids provide an efficient recycling mechanism for Si, in a similar way as observed for other nutrients. As human mowing management negatively impacts on the occurrence of tussocks, we have also made a first estimate of the potential effects of mowing on Si cycling. To answer these questions we selected several similarly sized tussocks of the Fibrous Tussock-sedge *Carex appropinquata* A. Schumach. and determined BSi content in different compartments. These data were compared to the BSi content of soil cores taken between tussocks and of soil cores from a managed parcel without tussocks.

2. METHODS

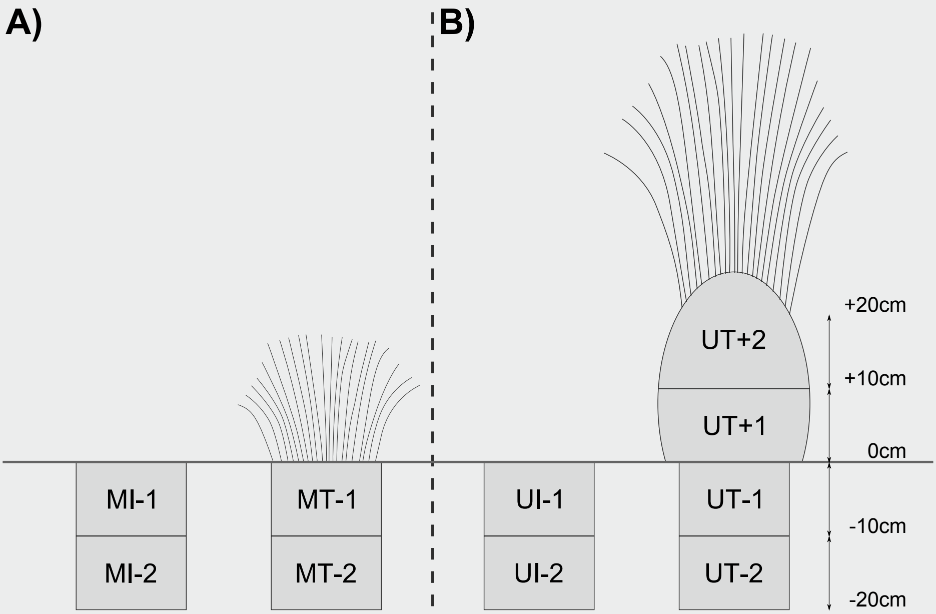
2.1 Site description

In the Biebrza National Park in Eastern Poland, low productive fen meadows have been mown traditionally for hay. However, abandonment began in the 1970’s. Between 1981 and 1983, ca. 30000 ha was still mown annually (Banaszuk 1994). Today less than 5000 ha of meadows within the park boundaries is mown or grazed. A characteristic zonation pattern of plant communities from the riverbed to the valley margins is still present. A more detailed description of the Biebrza valley can be found in (Wassen *et al.* 2006).

Our study plots are located near Rogozyn in the Upper Basin (N53°42’16”, E23°26’30”). Here, the river is 5 to 6 m wide. Discharges in early summer (June) range from 0.12 to 0.98 m^3 s^{-1} (De Doncker 2009). The soil consists of sandy/loamy peat (1 to 2 m deep, underlain by fluvial and eolian sand). The (former) hay meadows are bordered by a sandy moraine in the north (130 m ASL) and by the river Biebrza in the south (water level app. 119.5 m ASL). The water table throughout the season fluctuates between -30 and +10 cm. Shallow flooding occurs near the river during late winter-early spring (Struyf *et al.* 2009). Until the late eighties to early nineties all parcels in the study area were subjected to an annual mowing regime (end of June-July). However, annual mowing has been abandoned between 1991 and 1995 in some parcels. In this study we selected two sites in adjacent parcels, only differing in management regime (continued mowing regime vs. abandoned).

2.2 Tussock analysis

In the abandoned parcel eight well-developed tussocks of the Fibrous Tussock-sedge *C. appropinquata* were selected and sampled in late June 2007. Zero soil depth was arbitrarily defined as the mean height value of the space in between tussocks (here-after called inter-tussock). Soil depths were either positive (up into the tussock) or negative (down into the soil). We calculated the height for each tussock as the average elevation of the highest point of the tussock relative to four points evenly spaced around the base of the tussock. Each tussock was cut at zero soil depth using a serrated knife and divided into 0.1 m slices



↑ **Fig. 1** Sampling design for the determination of soil characteristics and BSi concentrations. Shaded areas indicate the tussock and inter-tussock parts sampled in the managed (A) and abandoned site (B). (M: managed, U: abandoned; I: inter-tussock, T: tussock).

starting from zero soil depth upwards. For each slice the surface of the lower and upper section was determined in order to calculate its volume using the formula for a truncated cone. One soil core (▯: 0.152 m, H: 0.2 m) was taken underneath every removed tussock. Additionally, eight ‘inter-tussock’ cores were taken in the abandoned parcel. In the mown parcel 16 soil cores with the same dimensions (eight with *C. appropinquata* shoots present, eight without) were taken. All cores were separated into 0.10 m high slices (Fig. 1A). All vegetation on top of a tussock or core was removed and sorted into living monocotyledons (mono), living moss (moss), living dicotyledons and equisetales (di_e), and dead material (dead), dried for 48 h at 70 °C and weighed.

After determining fresh weight, the core and tussock slices were subdivided into different parts. The first part was used for BSi-extraction after milling and the determination of moisture and organic content. A second part was used to determine soil pH and the concentration of extractable iron. Roots and stem parts were sorted from the third part, washed gently to remove adhering soil particles, dried at 70 °C for 48h, weighed and milled for BSi determination. For the purpose of this paper, bulk soil is defined as the entire soil matrix, encompassing soil minerals, soil organic matter (SOM) and living belowground plant biomass, occurring within and beneath tussocks. Net soil constitutes only of the soil minerals and SOM as the living belowground biomass (roots and rhizomes) has been removed. Roots and rhizomes are pooled and reported as belowground biomass.

2.3 Nutrient analysis

Extractable Fe was determined after an extraction of 20 g wet soil with 100 mL of a 0.5M ammonium acetate-EDTA (pH 4.65) solution (Cottenie *et al.* 1989). Fe concentrations were determined using a Thermo Iris ICP (Thermo Fisher Scientific, Waltham, USA). pH-KCl was determined in a 1:2.5 (w:v) solution (Houba *et al.* 1989). BSi was determined by alkaline extraction of 30 mg of homogenized dried soil in 1% Na_2CO_3 solution over a 5 hour period with subsamples taken at 3, 4 and 5 hours and neutralized as adapted by Conley & Schelske (2001). The extract was analysed for DSi and plotted against time. To correct for simultaneous dissolution of (silicate) minerals, the y-intercept was considered to be the BSi content. Plant material was only incubated for 4 hours. Extracts were analyzed for DSi concentration on a Thermo Iris ICP (Thermo Fisher Scientific, Waltham, USA). Organic content was determined according to Houba *et al.* (1989).

2.4 BSi-stock calculation

To determine the stock of biogenic silica in the upper 0.20 m of the soil, the tussocks and the aboveground vegetation, a digital elevation model (DEM) of a 5 × 5 m plot was made after removing the aboveground vegetation. The plot was scanned using a FARO Focus 3D 120 scanner (Faro Technologies UK Ltd; Coventry; United Kingdom) with a scan resolution of 6136 mm per 10 m.

It was installed in two opposite corners of the site at a height of 1.6 m above ground level. For each scanner location an XYZ cloud of points was stored. Both point clouds were merged using the software-package Faro Scene 4.6 (Faro Technologies UK Ltd; Coventry; United Kingdom). The obtained image was further processed using ArcGIS 10 (Esri, Redlands, USA). With this DEM, the ratio of the surface occupied by tussocks to the total plot surface and the tussock volume above the zero soil level could be determined and was used to calculate BSi-stocks. In the mown parcel, the surface ratio was determined using vegetation relevés. A boot-strap randomization with 10000 simulations to calculate 95% confidence intervals of the BSi-stocks was done using the R-package boot (Canty & Ripley 2011).

2.5 Data analysis

A linear mixed-effect model with post-hoc comparison was used to test differences in BSi-concentrations or other soil properties among tussock compartments. Data were square-root, log or arcsin-transformed when necessary to improve homogeneity of variances. Means and standard errors shown in Fig. 3 and 4 were calculated on transformed data and then back transformed to aid visual representation. Analyses were performed with R 2.10.1 (R Development Core Team 2009). Linear mixed-effect models were run using the nlme package (Pinheiro *et al.* 2010) with Tukey's pairwise comparison following Hothorn *et al.* (2008).

3. RESULTS

3.1 Soil properties

In the mown parcel, soil bulk density was highest in the deepest layers (layer U-2; 210 mg cm^{-3}) but did not differ significantly between patches with and without tussock-forming vegetation. In the abandoned parcel, there were no big differences in soil bulk density among the different soil compartments in layer -1 and -2. However, both upper tussock layers showed a significantly lower bulk density (120 mg cm^{-3}, Fig. 2A). Compared to soil bulk density, belowground biomass showed an opposite pattern. In the deepest layer below well-developed tussocks (UT-2), belowground biomass was only slightly higher than in inter-tussock. In the upper tussock layer (UT+2), belowground biomass was highest and accounted for almost half of the bulk density (Fig. 2B). Both moisture and organic matter content were high (both around 80%) and showed little variation. Only in the upper tussock layer they were respectively considerably lower and higher than in the other compartments (Fig. 2C, D). pH ranged from 5 to 6 and showed relatively little variation among the different compartments. Yet, in well-developed tussocks pH gradually increased from the deepest to the higher layers but showed a clear drop in the upper tussock layer (UT+2; Fig. 2E). In between well-developed tussocks, $Fe^{2+/3+}$ concentrations in the upper and lower soil layer did not differ. In the mown site, concentrations in the upper soil layer were only slightly, but not significantly, higher than in the lower soil layer (Fig. 2F).

3.2 Aboveground Vegetation

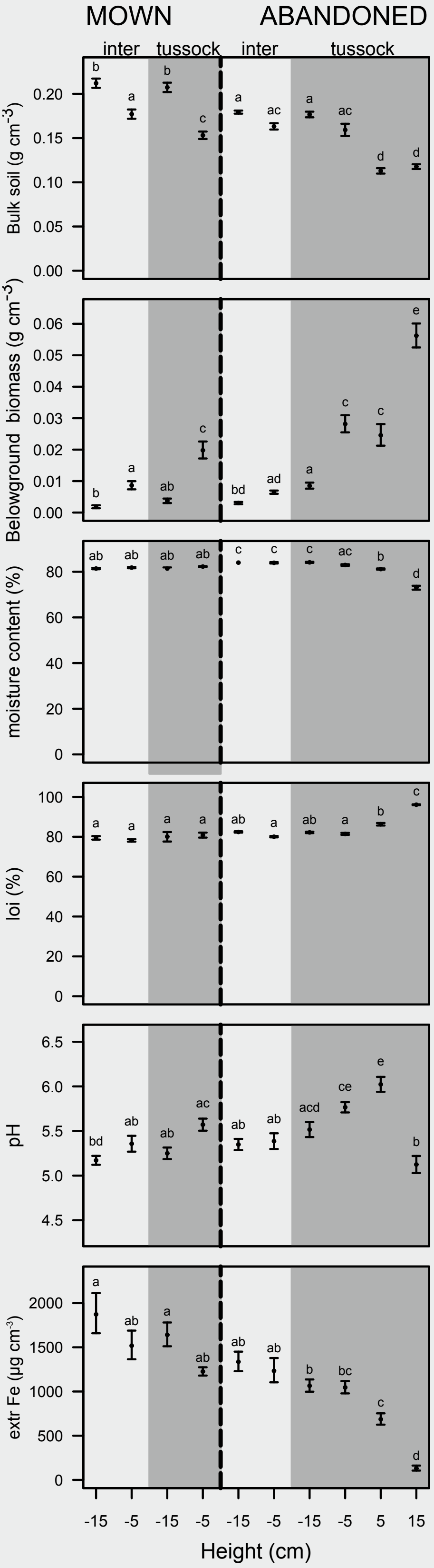
Living aboveground biomass was highest on top of well-developed tussocks (2800 g m^{-2}) in the abandoned parcel, with monocotyledons (mainly *C. appropinquata*) contributing more than 98% (Fig. 3A). Here, aboveground biomass production in inter-tussock was almost negligible. In comparison with the abandoned parcel, aboveground biomass production in tussock-patches in the managed parcel was considerably lower (912 g m^{-2}). Furthermore, monocotyledons only accounted for 75%. In patches without *C. appropinquata* shoots, aboveground biomass was reduced to 195 g m^{-2} with mosses being most dominant (80%). In the managed parcel dominance of *C. appropinquata* was lower. Especially in the inter-tussock areas monocotyledons encompassed several other grass and sedge species (e.g. *C. nigra*, *Festuca rubra*, *F. pratensis*, …).

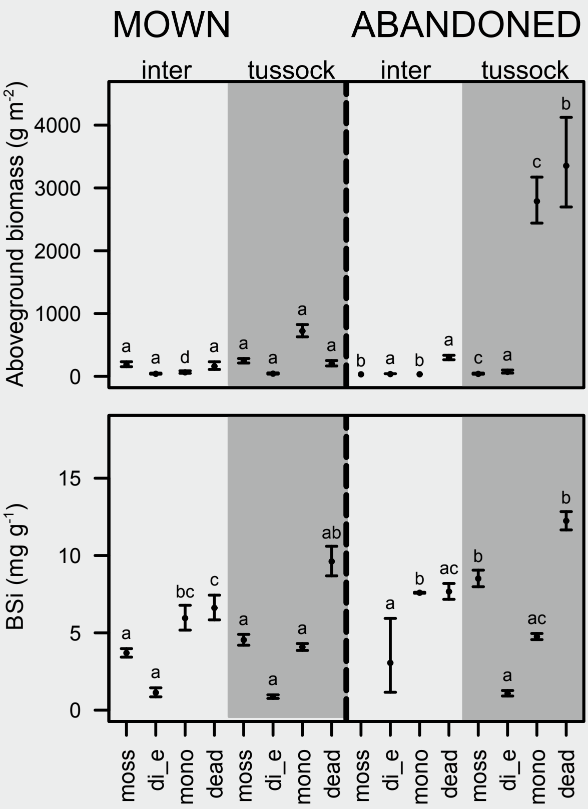
3.3 BSi

In the mown site as well as in between tussocks in the abandoned site, BSi concentrations in the bulk soil were higher in the upper than in the lower soil layer. Within and below well-developed tussocks, a clear gradient in BSi concentrations could be observed. Higher concentrations in the upper tussock-layers were partly due to the higher belowground biomass combined with strongly increasing BSi concentrations in this biomass compartment. This is confirmed by BSi concentrations in the net soil, as these, when expressed volumetrically, did not increase within well-developed tussocks (Fig. 4A, B). BSi concentrations were highly variable among vegetation components but were highest in the dead material on top of well developed tussocks (12 mg g^{-1}; Fig. 3B).

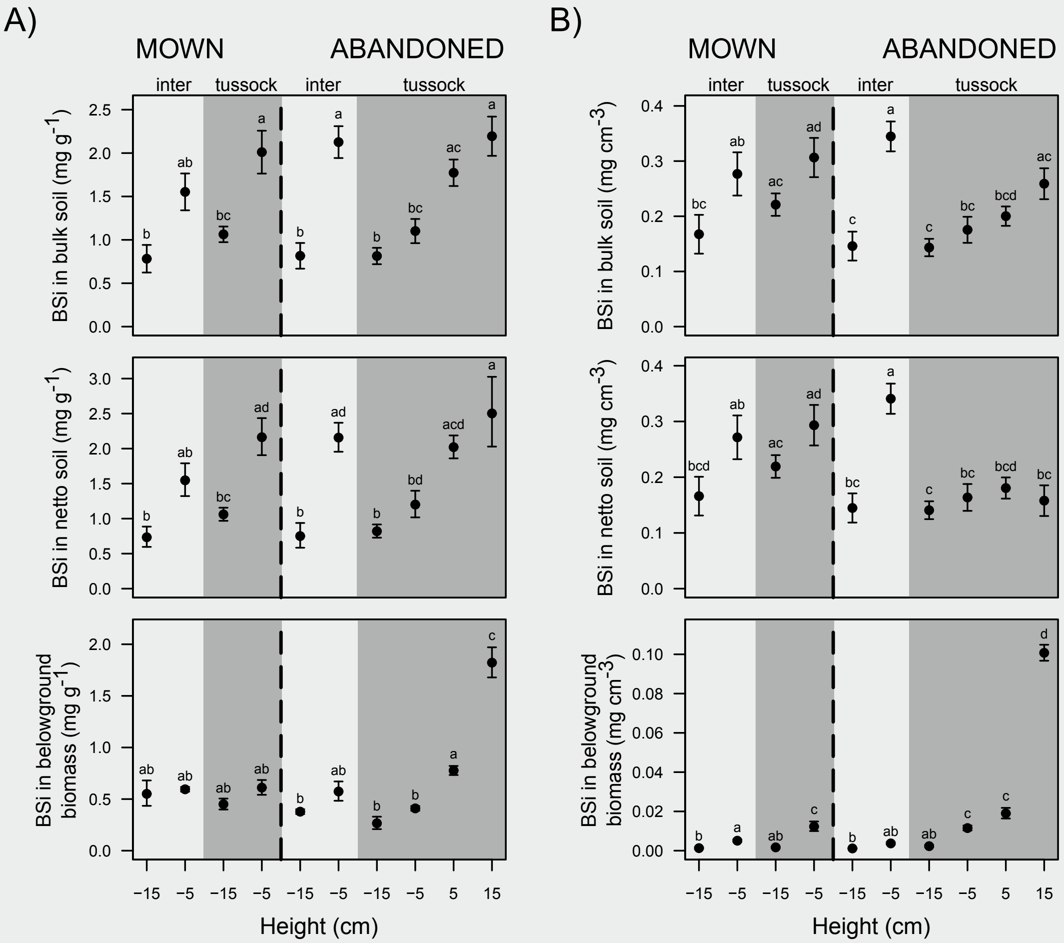
The combined BSi stock for the upper 0.20 m of the soil and the aboveground biomass was considerably, although not significantly ($p = 0.1153$), higher in the abandoned site compared to the mown site (Fig. 5). The difference was 38%, 1450 vs. 1050 kg SiO_2 ha^{-1}. Yet, the BSi stock in the bulk soil material was roughly the same in both sites. The difference in BSi-stock between both management types was mainly attributed to the BSi stored in the dead material on top of well-developed tussocks and to a lesser extent to the living aboveground biomass on top of these tussocks (together $p < 0.001$).

→ **Fig. 2** Soil parameters in the sampled tussock and inter-tussock compartments. Significant differences among compartments ($p < 0.05$) are indicated by superscript letters above the flags. Data were square-root, log or arcsin-transformed when necessary to improve homogeneity of variances. Means and standard errors shown were calculated on transformed data and back transformed to aid visual representation. For location of the sampled compartments, see Fig. 1.

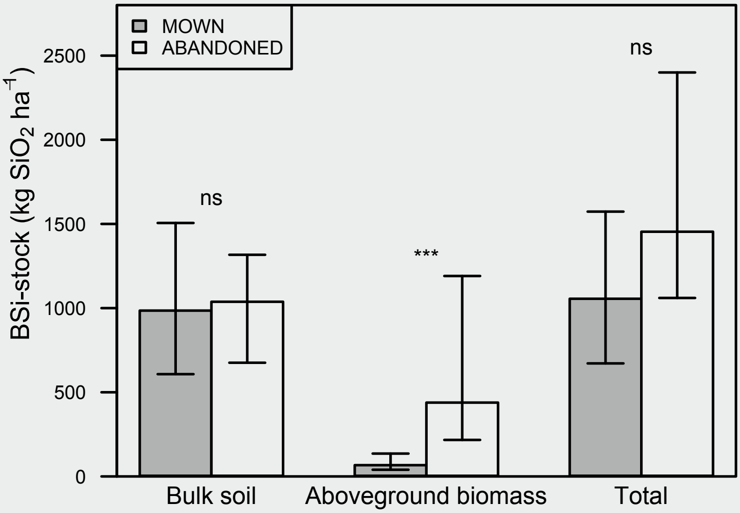




↑ **Fig. 3** Top: Aboveground biomass, split in moss (living moss), di_e (cotyledons and equisetales), mono (monocotyledons), dead (dead material); Bottom: BSi-concentrations in the aboveground biomass. Significant differences among compartments ($p < 0.05$) are indicated by superscript letters above the flags. Data were square-root transformed when necessary to improve homogeneity of variances. Means and standard errors shown were calculated on transformed data and back transformed to aid visual representation. For location of the sampled compartments, see Fig. 1.



↑ **Fig. 4** BSi-concentrations in the sampled tussock and inter-tussock compartments. Left-panel: gravimetrical BSi-concentrations for the bulk soil, net soil and belowground biomass. Right-panel: volumetric BSi-concentrations for the bulk soil, net soil and belowground biomass. Significant differences among compartments ($p < 0.05$) are indicated by superscript letters. Data were square-root transformed when necessary to improve homogeneity of variances. Means and standard errors shown were calculated on transformed data and back transformed to aid visual representation. For location of the sampled compartments, see Fig. 1.



↑ **Fig. 5** BSi-stock in the mown and abandoned site. Confidence-intervals (95%) were calculated using a bootstrap-simulation with 10000 permutations. Significant differences in BSi-stock between management types are indicated with superscript text (ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

4. DISCUSSION

4.1 Silica patterns

BSi concentrations in wetland plant species in general range between 0 and 70 mg g^{-1} dry weight, with extremes up to 180 mg g^{-1} (e.g. Struyf *et al.* 2005, Struyf & Conley 2009). *Poaceae* and *Cyperaceae* are well represented in the higher concentration ranges, as both are well-known silica accumulators containing large amounts of Si relative to their dry weight (>10 mg g^{-1}; Ma *et al.* 2001). Our values fit within the lower range of concentrations previously described for *Poaceae* and *Cyperaceae*. This is most likely explained by the seasonal variability in plant BSi concentration (Struyf *et al.* 2005), which usually follows a unimodal distribution throughout the growing season. The lowest concentration is observed at the beginning of the growing season, while Si concentration in plant tissues increases gradually during summer and reaches maxima in early autumn. The rather low concentrations we observed can be explained by the sampling date situated in early summer.

Soil BSi concentrations in this study (layers -2 and -1) coincide with values observed by Struyf *et al.* (2009), who sampled the upper 0.30 m of soil along several transects at the same site. Soil BSi concentrations at the sampling site are low compared to previous studies in tidal wetlands (Struyf *et al.* 2005) and especially compared to boreal peat bogs (Struyf *et al.* 2010). However, despite these low concentrations, clear control of BSi on available DSi in the soil water was observed (Struyf *et al.* 2009). Low concentrations of BSi in our site may be related to the generally nutrient-poor character of the upper ranges of the Biebrza river (Olde Venterink *et al.* 2009).

4.2 Silica patterns in tussocks

The novelty of our results lies within the analyses of BSi patterns within tussocks. To our knowledge, a similar analysis has never been performed, despite the importance of grasses and sedges as silica accumulators in ecosystems, and the important control wetland grasses and sedges can exert on terrestrial – aquatic Si fluxes (Struyf & Conley 2009, Struyf *et al.* 2011). BSi concentrations, expressed gravimetrically, showed a clear positive gradient within tussocks. Concentrations just below fully developed tussocks are significantly lower than in the adjacent intertussock patches. This indicates that plants effectively lift up BSi from the wetland soil into the tussock. Hence, a large part of the Si in tussock dominated plots is included in the living and dead aboveground biomass, as compared to managed plots without tussocks. Dissolution of BSi from dead aboveground material is determined by physical processes, especially by contact with water and the frequent refreshing of the solution (e.g. Struyf *et al.* 2007). As the upper part of the tussocks is situated well above the water table and has thus a drier character, this potentially leads to in BSi enrichment in the upper tussock layers.

Dead shoots had higher BSi contents than living shoots, especially on top of well-developed tussocks. The difference between old (dead) and young (living) shoots is partly explained by the early sampling date as explained above. Next, typically 30 to 90% of a leaf's nitrogen and phosphorus pool is resorbed during leaf senescence (Aerts & Chapin III 2000). Furthermore, nitrogen and organic carbon are more labile plant elements than BSi (Eleuterius & Lanning 1987) in standing dead shoots. As the more labile components leach from the plant tissue during early decay processes, BSi content of standing dead shoots increases relatively. As long as dead shoots remain on top of a tussock, BSi is detached from physical dissolution processes. Hence, dead aboveground biomass on tussocks has a higher BSi content than dead biomass in between tussocks. As long as the dead biomass on top of tussocks is still standing straight or merely hanging down, it indeed faces only limited contact with water, compared to litter in between tussocks which is kept moist continuously. Once dead shoots get detached from the tussock, BSi dissolution goes fast due to the intense contact with water (Struyf *et al.* 2005).

The positive gradient in bulk gravimetrical BSi concentrations was attributed to an increase of both net soil and plant material BSi concentrations in the tussocks. The annual burial of dead plant material on top of a tussock leads to a stock of BSi which only slowly dissolves, hence the decreasing concentration gradient deeper down the tussock. Most of the dissolved Si is likely re-acquired by the growing biomass during the growing season, keeping the concentration gradient intact. However, outside the growing season, BSi that dissolves will flow to the wetland soil again, which is most likely compensated for by the active uplift of BSi from the adjacent intertussock soil to the tussock during the growing season again, as observed for other nutrients (Chapin III *et al.* 1979). Hence, various processes occur that keep the concentration gradient intact. When expressed volumetrically, this gradient is less steep or even absent for concentrations in net soil. The observed increase in BSi stored in biomass, expressed volumetrically, can not only be explained by the increased gravimetric BSi concentrations, but also by the higher levels of plant biomass in the upper layers of a tussock.

Silica accumulation can provide plants with several competitive advantages (Epstein 2001, Massey *et al.* 2007). In our study, BSi accumulation within tussocks and sedge tissues should result in increased DSi availability, as BSi and DSi concentrations are usually tightly coupled (Struyf *et al.* 2009). This strengthens the hypothesis of Struyf & Conley (2012) that graminoid tussocks alter Si availability (as observed for other nutrients, e.g. Jonasson & Chapin III 1991) and as such create their "private" Si stock, making this key nutrient more easily available by efficient recycling.

4.3 Tussocks and the silica sink in wetlands

Wetlands are generally regarded as hot-spots for nutrient cycling. Likewise, they are also important processors in the biogeochemical BSi cycle, as more and more research emphasizes. In tidal wetlands, emergent vegetation (e.g. reed) takes up large amounts of DSi, consequently stored in the aboveground vegetation, turning the system into hot-spots of active BSi cycling in estuaries (e.g. Struyf *et al.* 2006, Jacobs *et al.* 2008) and increasing resilience of estuarine ecosystems during periods of pelagic silica limitation. A similar hypothesis has been proposed for boreal wetlands, where Struyf *et al.* (2010) found enormous stocks of BSi in the upper soil layers (up to 11% of dry weight). An inverse modelling approach showed that, despite being a permanent Si sink, vegetation cover, at first site paradoxically, leads to increased silica fluxes compared to a system where wetlands have been drained and mostly purely geological processes control the Si cycle.

Similarly, as we show, in grasslands dominated by tussocks, Si is translocated from soil underneath and between tussocks into the BSi rich tussock top. This not only results in a vertical gradient within tussocks but also in a higher total stock of BSi in tussock dominated grasslands compared to annually mown sites. As was the case in boreal wetlands, the system consumes DSi delivered by the groundwater and becomes a net sink, *in casu* mainly in the tussock dead biomass. In pristine riverine wetlands, hydrological gradients are the main drivers of differences in vegetation composition (e.g. Wassen *et al.* 1990, Grootjans *et al.* 1996). As anthropogenic factors became an important driver as well, in present times the management of meadows is either heavily intensified or abandoned. Mowing cessation in wet grasslands often leads to rapid tussock formation, and later shrubs encroachment (Preiss 1982, Fossati & Patou 1989, Diemer *et al.* 2001). We here show that this increases silica storage capacity of the wetlands by 38%. The eventual delivery of this silica to the river will depend on the trade-off between DSi annually lost from the tussocks, and the DSi net taken up from the soil into the tussock. If these are in equilibrium, the active uplift of soil Si into vegetation in tussock wetlands compared to managed wetland, and subsequent leakage from the tussock, will probably result in actively increased export potential to the river as compared to managed sites. In addition, in managed sites, BSi is translocated from the system into an agricultural pathway, potentially further diminishing exchange capacity for Si between river and wetland. Mowing of Si rich grasses is a largely understudied topic, of potentially major importance in global and local Si cycling (Vandevenne *et al.* 2012).

4.4 Conclusion

Our results show that tussock development causes a patch-like distribution of BSi stocks in wetlands. While in a managed wetland BSi is uniform over the surface layers, development of tussocks strongly interferes with this pattern. A mosaic of biogenic silica richer intertussock soils and biogenic silica poorer soils under tussocks develops. This is the result of the active uplift of the Si by the tussock forming species into the tussock and the aboveground biomass.

Tussocks provide relatively dry spots within the wetland, where more optimal growing conditions occur (Crain & Bertness 2005). Hence, other species can compete with grasses for these more optimal growth locations. We suggest that silica patterns within tussocks reflect the efficient recycling of Si by the tussocks grasses: as Si is an effective enhancer of grasses competitiveness, this provides grasses with an advantage over other species. They create hot-spots of a key nutrient they are best at recycling.

More and more results show that wetlands play a key role in mediating Si fluxes between the aquatic and terrestrial environment. Our study shows that biogeochemistry and the availability of Si changes in tussock-dominated wetlands compared to managed wetlands without tussocks. Tussocks will have an effect on the role of wetlands as silica hot-spots and biogenic Si sinks near rivers. This implies that future studies should focus on quantifying the effect of tussock development, and human management, on system scale BSi storage.

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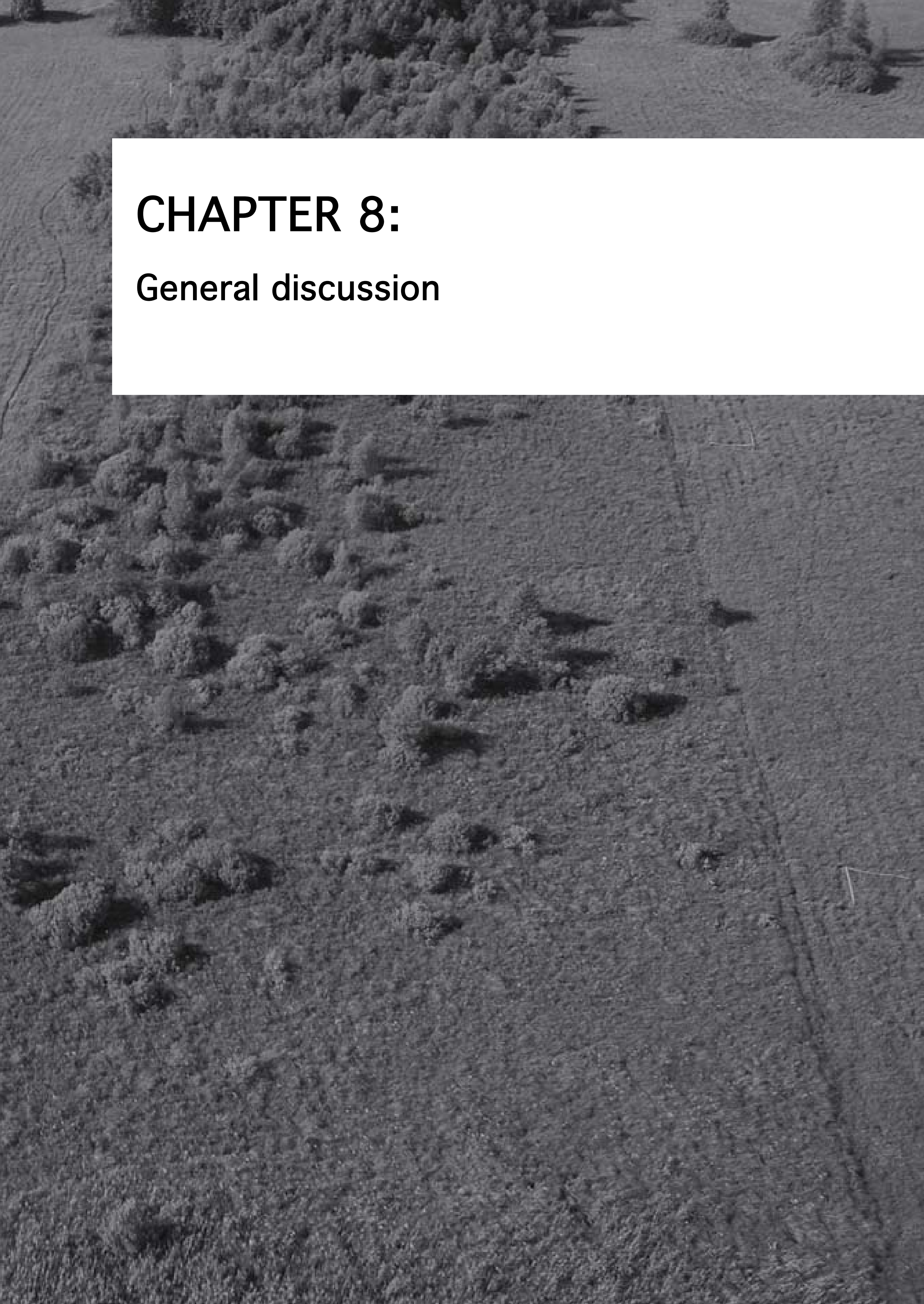
CHAPTER 8:

General discussion



GENERAL DISCUSSION

With this work, we aimed to further expand the understanding of the factors governing species and functional diversity in riparian wetlands. In doing so, the effects of hydrology and competition were the main focus of this study, together with the interactions of human management and these factors. Moreover, the consequences of these factors on Si cycling in riparian wetlands were explored.

SPECIES DIVERSITY IN RIPARIAN WETLANDS

Of the different factors known to affect species composition in riparian wetlands, we focused on two factors (hydrology and light availability) in two different settings. In **Chapters 2** and **3**, their importance for driving early community assembly was studied using a mesocosm approach while their effects on species patterns following succession in riparian hay meadows were studied in **Chapter 4**.

Using a mesocosm approach (**Chapter 2**), the strong filtering effect of an established canopy on community assembly was shown. At least in the first year, it almost completely hampered species establishment. This strong effect was probably due to the high light attenuation imposed by the grass canopy. Yet, effects of e.g. increased root competition could not be excluded. Early summer mowing slightly reduced this filtering effect and, due to the temporal alleviation of competition, allowed for the establishment of a few species. Waterlogging, leading to oxygen depletion, often has detrimental effects on many plants, including typical wetland species. In our study, it was proven to be a stress factor, but only resulted in decreased plant growth rates. It did not affect germination or species richness directly. On the contrary, in the absence of a closed canopy at the onset of the experiment, waterlogging allowed for the highest species richness. Decreased growth rates under waterlogged conditions led to higher light availability and presumably lower competition for light between the establishing plants. Hence, the simultaneous response of species to waterlogging and competition resulted in a trade–off, leading to a strong synergic filtering effect when both types of stress occured simultaneously. The first year results indicate a hierarchical model, with canopy effects acting as a stronger filter than waterlogging, contrary to the results of Lenssen *et* *al.* (2003). Yet, the relative importance of waterlogging and initial vegetation cover as drivers of species community composition changed during the consecutive years of the mesocosm experiment (**Chapter 2bis**). This stresses the need for long–term research in community ecology as interpreting single year results might lead to wrong conclusions. In **Chapter 3** we showed how waterlogging strongly affected the composition of both vegetation and soil microbial community in riparian systems. Initial grass canopy cover on the contrary only had a pronounced effect on the composition of the aboveground vegetation. It was considered as having a large priority effect on wetland plant community assembly (Körner *et al.* 2008). Although this priority effect is mainly visualized aboveground, it is also reflected to some degree in the microbial community, through e.g. altered effects of root exudates, litter quality, etc. However, the effects of plant species richness on soil microbial community composition still remain unclear (Gastine *et al.* 2003) and probably depend on the functional composition of the aboveground community (Spehn *et al.* 2000, Stephan *et al.* 2000, Ladygina & Hedlund 2010, but see Marshall *et al.* 2011). Furthermore, the apparent lower response of the soil microbial community to vegetation composition can possibly be attributed to a general time–lag in the response of these communities to changed environmental conditions (Groffman *et al.* 1996, Hedlund *et al.* 2003, Habekost *et al.* 2008, Eisenhauer *et al.* 2010).

The interplay between hydrology and light availability as drivers of species composition during secondary succession in riparian systems was studied in a different set–up (**Chapter 4**). Following abandonment, the environmental conditions experienced by plants change. Litter accumulated and vegetation height increased with a subsequent reduction in light availability. Again, increased competition for light strongly affected both species richness and eveness, while the hydrological gradient, despite its link with aboveground biomass production, had no significant effect.

Generally, this study has shown that light competition acts as a main environmental filter during species assembly in riparian wetland vegetation and hence confirms the results of earlier studies (e.g. Aerts 1999, Jensen & Meyer 2001, Kotowski & van Diggelen 2004, Edelkraut & Güsewell 2006, Kotowski *et al.* 2006, Schrautzer & Jensen 2006). However, other factors should not be neglected when studying community composition in riparian systems. As shown in this thesis, waterlogging was an almost equally important driver of community composition in later stages of community assembly and was shown to determine species richness. These results add to the knowledge of riparian restoration and conservation. Human management, through its effects on light availability, directly influences community composition. However, restoration goals can only be reached seldom if other environmental constraints are not taken into account. These constraints are numerous. Both site and landscape conditions (hydrological regime, soil constitution and nutrient content, seed availability, historical land use, …) clearly affect the feasibility to achieve restoration objectives.

FUNCTIONAL DIVERSITY

Even at the same site, seedlings can be subject to different light regimes and soil resource availability than adult plants. This can be attributed to their small size. In riparian wetlands, species assembly patterns and traits related to germination and seedling establishment were clearly linked during early community assembly (**Chapter 2**). Both stages are considered as bottlenecks for species recruitment (Grubb 1977, Shipley *et al.* 1989). Although both wetness and light availability affected germination, only few species were directly eliminated at this stage. The main filtering obviously took place during the next phase, i.e. establishment, as all species which germinated under a dense grass canopy failed to persist. Nevertheless, a limited establishment could be observed when mowing created a "light window". The few species which established under a dense canopy, all showed a combination of traits typical for a stress–tolerant plant strategy, allowing them to minimise resource requirements and survive in suboptimal conditions. On bare ground, seedling competition among seedlings was substantial, unless a stress factor (i.e. waterlogging) limited inter–specific competition. Thus, traits responsible for rapid establishment and out–competing neighbours appear more important here than those responsible for shade tolerance (Keddy *et al.* 1994, Stockey & Hunt 1994). This stresses the importance of priority effects during community assembly in grasslands as observed by Stockey & Hunt (1994) and Körner *et al.* (2008). In waterlogged soils however, the importance of priority effects decreased and most species were able to establish.

Once established, plants face other environmental conditions than during the seedling stage. Hence, plant persistence might depend on other than germination and establishment traits. Yet, in the consecutive years of the mesocosm experiment (**Chapter 2bis**), species and trait patterns remained interrelated, which further stresses the importance of the germination and establishment phase for determining diversity patterns (Grime 1979, Henry *et al.* 2004).

In riparian wetlands secondary succession strongly affects species composition, and hence, trait distribution. The plant traits related to the environmental conditions in **Chapter 4**, were all significantly related to mowing cessation while only half of them were related to the hydrological–productivity gradient. This emphasizes the dominant effect of management cessation compared to hydrology as drivers of functional composition of the sites studied. Consequently, most traits were related to a plant's ability to compete for light, thereby confirming the earlier findings of Kotowski & van Diggelen (2004) and Schrautzer & Jensen (2006). These authors showed that light competition is a major factor determining species zonation in fen systems. In a recent study across a wide range of northwest European ecosystems, Douma *et al.* (2012) also found succession–induced trait shifts to be driven by an universal response to light availability and a specific response depending on initial abiotic conditions.

In wetlands, plants dispose of a wide range of traits to grow under oxygen depleted conditions following waterlogging (Brix & Sorrell 1996, Pezeshki 2001). One of the principal adaptations in different *Carex* species is the development of aerenchym to supply their roots with oxygen. Tussock growth, a commonly observed growth form of sedges, forms a structural escape mechanism to avoid detrimental effects of soil waterlogging (**Chapter 6**). In fen and hay meadows, mowing cessation leads to increased tussock development. Through the physical modification of their abiotic environment, tussock–forming plants created a new structural state (Jones *et al.* 1997). This structural change modified resource availability as the creation of an aerobic habitat altered biogeochemical cycles, which eventually lead to higher nutrient availability within these tussocks. This, on its turn, resulted in a tripled above-ground biomass production. Such a structural change is therefore beneficial for the tussock–forming plant species. Furthermore, *C. appropinquata* acted as an ecosystem engineer by changing its environment. Yet, whether its effect on other species is positive or negative clearly depended on the evaluation scale.

CONSEQUENCES FOR THE SI CYCLE IN RIPARIAN WETLANDS

In **Chapter 5**, local hydrology and vegetation composition (i.e sedge and grass dominance) jointly controlled BSi storage in riparian hay meadows. Human management (i.e. mowing), through its effects on vegetation composition and tussock development, strongly affected BSi, and hence DSi export to the river. In addition, BSi in managed sites is translocated from the system into an agricultural pathway as hay was removed annually. This potentially decreases the exchange capacity for Si between riparian wetlands and rivers. The importance of mowing of Si rich grasses on global and local Si cycling remains largely unknown (Vandevenne *et al.* 2012). The potentially far–reaching consequences of human management on Si cycling were shown in **Chapter 7** where tussock development, following secondary succession, caused a patch–like distribution of BSi stocks in wetlands. Here, Si was translocated from the soil underneath and between tussocks into the BSi rich tussock tops. This not only resulted in a vertical gradient within tussocks but also in a higher BSi stock in tussock–dominated grasslands compared to annually mown sites. Due to mowing cessation, the silica storage capacity of this site increased with 38%. This could mainly be attributed to the non–removal of aboveground biomass. This stresses the importance of human activities for Si cycling in soils and for the land–ocean Si flux (Clymans *et al.* 2011).

In general, the impact of vegetation composition and human management on BSi storage, and subsequent DSi export, should be taken into account during the planning process of water retention and water storage areas (e.g. through conversion of former arable land into floodplains), together with biogeochemical constraints (Lamers *et al.* 2006), possible effects on nutrient retention (Olde Venterink *et al.* 2006), … in setting restoration goals.

SOME REMAINING QUESTIONS

Our understanding of the ecology, functioning and restoration of riparian wetlands in general, has greatly increased in the last decades. In this thesis, we contributed to this knowledge. Yet, several knowledge gaps still remain and require more attention in future research. First of all, the importance of above–belowground interactions and their interplay with other factors, in driving community composition in riparian systems remains unclear. Based on research in other ecosystems (e.g. Kardol & Wardle 2010), these interactions could also play a major role in the functioning and restoration potential of riparian wetlands.

Next, a further development of statistical methods that can link environmental factors and traits in multi–species studies is needed. Current available multivariate statistical methods (e.g. RLQ, Fourth Corner method) are usually based on a correlative link between trait and environment variables. To understand variation patterns in species density, a multivariate approach will be necessary as a one–to–one approach is not sufficient. This is due to the often high number of interacting traits and environmental variables. As such, the availability of predictive statistical methods, combining trait and environmental information in a multivariate approach, would be a useful tool to further expand our knowledge on processes like community assembly, secondary succession, … Furthermore, many of the currently used multivariate statistical methods (e.g. RLQ) do not include a method to extract only those traits from a wider set which describe an ecosystem response to a given environmental gradient best. Recent studies proposed methods to identify the most suitable traits for plant functional trait analyse (Bernhardt-Römermann *et al.* 2008) or the most suitable multivariate method to assess species and community functional responses to environmental gradients (Kleyer *et al.* 2012). Yet, other methods using a mixed–model approach would be useful.

Most trait–based approaches consider intraspecific trait variability as negligible. However, recent work (e.g. Albert *et al.* 2011) has underlined the need to integrate variation at both the intraspecific as well as interspecific level. New methods (e.g. Violle *et al.* 2012), including intraspecific trait variability, will facilitate the shift from species-based to individual-based community ecology and lead to a more predictive ecological theory.

Finally, a growing number of studies shows that riparian wetlands play a key role in mediating Si fluxes between the aquatic and terrestrial environment. Future studies should thus focus on quantifying the effects of vegetation, hydrology, and human management on ecosystem scale BSi storage. Furthermore, to enhance our understanding of wetland Si dynamics and the sink–source function of riparian systems, exchange studies are a necessary prerequisite.

The above–mentioned topics are only a few of the numerous topics which deserve more attention in future research.

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Summary
Dankwoord
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SUMMARY

The title "Composition and functional diversity in riparian wetlands: interactions between hydrology and competition" gives away the main topic of this manuscript. Of the different factors known to affect species composition in riparian wetlands, we focused on two factors (hydrology and light availability) in two different settings.

Using a mesocosm approach that focused on the effects of waterlogging, initial canopy cover and summer mowing, we showed how single stress factors can enhance both plant species richness and functional diversity by limiting interspecific competition. Yet, a synergic interaction of different stress factors can lead to reduced richness. Even in the third year of the experiment, establishment success was still clearly linked to seedling traits. This emphasizes the importance of the germination and establishment phase for the determination of community diversity patterns. Compared to the first year, the linkage between waterlogging and seedling traits increased during consecutive years of the experiment. This is supportive for a hierarchical model of environmental filters, where habitat filtering is considered to be dominant over competition. Next, the experimental factors clearly affected the composition of the microbial communities. Of these, waterlogging had the largest effect. Interestingly, the effect of grass canopy cover was less pronounced in the microbial than in the floristic community.

The interplay between hydrology and light availability as drivers of species composition during secondary succession in riparian systems was studied in a different set–up. Following abandonment, the environmental conditions experienced by plants in riparian hay meadows change as litter accumulated and vegetation height increased. The subsequent increased competition for light strongly affected both species richness and evenness, while the hydrological gradient, despite its link with aboveground biomass production, had no significant effect. Through its effects on vegetation composition, mowing cessation also affected the storage of biogenic silica (BSi) in a riparian wetland. This supports the hypothesis that wetlands are important ecosystems in the biogeochemical cycling of silica. Next, mowing cessation in fen and hay meadows often leads to tussock development. Tussock growth, a commonly observed growth form of sedges, forms a structural escape mechanism to avoid detrimental effects of soil waterlogging. The creation of an aerobic habitat ultimately resulted in a higher nutrient availability within these tussocks and a tripled aboveground biomass production, turning this structural change highly beneficial. Finally, tussock development caused a patch-like distribution of BSi stocks in riparian wetlands. While BSi was uniformly distributed over the surface layers in a managed wetland, tussock development strongly interfered with this pattern. A mosaic of BSi richer inter-tussock soils and BSi poorer soils under tussocks developed, which resulted from the active uplift of Si by the tussock forming species into the tussock and the aboveground biomass. Hence, tussocks have an effect on the role of wetlands as silica hot-spots and biogenic Si sinks near rivers.

SAMENVATTING

De titel “Gemeenschapssamenstelling en functionele diversiteit in beekdallandschappen – interacties tussen hydrologie en competitie” verklapt reeds het hoofdthema van dit manuscript. Verschillende factoren beïnvloeden de soortensamenstelling in beekdalen. In dit doctoraat hebben wij gefocust op twee factoren (hydrologie en lichtbeschikbaarheid) en dit in twee verschillende omgevingen.

Met behulp van een mesocosmos opstelling waarbij gefocust werd op de effecten van een waterverzadigde bodem, de aanwezigheid van een dense grasmat en zomer maaien, konden we aantonen hoe afzonderlijke stress factoren zowel soortenrijkdom als functionele diversiteit kunnen bevorderen door interspecifieke competitie te beperken. Een synergetische interactie van verschillende stress factoren kan de soortenrijkdom echter reduceren. Zelfs in het derde jaar van het experiment was het vestigingssucces nog altijd duidelijk gerelateerd met de eigenschappen van zaailingen. Dit benadrukt het belang van de kiemings- en vestigingsfase voor het vastleggen van de diversiteitpatronen in plantengemeenschappen. In vergelijking met het eerste jaar, nam de relatie tussen een waterverzadigde bodem en zaailingeigenschappen toe tijdens de latere jaren van het experiment. Dit ondersteunt het hiërarchische model van omgevingsfilters, waarbij een habitatfilter, vergeleken met een competitiefilter, als dominant aanzien wordt. Daarnaast hadden de experimentele factoren een duidelijk effect op de samenstelling van microbiële gemeenschappen. Waterverzadiging bleek het grootste effect te hebben, terwijl het effect van een dense grasmat op de samenstelling van de gemeenschappen veel minder uitgesproken was voor de microbiële dan voor de plantengemeenschap.

De wisselwerking tussen hydrologie and lichtbeschikbaarheid als drijvende factoren van soortensamenstellingen tijdens secundaire successie in beekdalen werd bestudeerd in een andere set-up. Wanneer hooilanden verlaten worden, wijzigen de omgevingscondities die planten ervaren. Strooisel hoopte zich immers op en de vegetatiehoogte nam toe. De daaropvolgende toename in licht competitie had een grote invloed op zowel soortenaantallen als diversiteit. Ondanks de relatie met de bovengronds biomassa productie had de hydrologische gradiënt geen significant effect. Door het effect op de vegetatiesamenstelling,

beïnvloedde het wegvallen van het maaibeheer ook de opslag van biogeen silicium (BSi) in een beekdal. Dit vormt een ondersteuning voor de hypothese dat wetlands belangrijke ecosystemen zijn in de biogeochemische cyclering van silicium. Daarnaast leidt het wegvallen van maaibeheer in natte hooilanden vaak tot de ontwikkeling van zeggepollen. Polvorming is een structureel ontsnappingsmechanisme om de negatieve effecten van een waterverzadigde bodem te vermijden. De creatie van een aëroob milieu resulteert finaal in een hogere nutriëntenbeschikbaarheid in deze pollen en een drievoudige toename van de bovengrondse biomassa. Tot slot leidde polvorming tot een vleksgewijze verdeling van de BSi-stock in beekdalen. In een gemaaide hooiland was BSi immers uniform verdeeld over de oppervlaktelagen, maar polvorming interfereerde sterk met dit patroon. Een mozaïek van BSi-rijkere bodems tussen pollen en BSi-armere bodems onder pollen ontwikkelde zich, wat kwam door de actieve opname van Si door de polvormende soort. De aanwezigheid van pollen heeft dus een invloed op de rol van wetlands als silicium hotspots and BSi-sinks.

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