Shift in plant-soil interactions along a lakeshore hydrological gradient

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Abstract

Wetlands occupy the transitional zone between aquatic and terrestrial systems. Hydrological conditions have significant influence on wetland plant communities and soil biogeochemistry. However, our knowledge about plant-soil interactions in wetlands along hydrological gradients is still limited, although it is crucial to guide wetland management decisions and to adapt, whenever possible, hydrological conditions to the different plant communities. To this aim, we related vegetation composition, plant functional traits, soil physicochemical properties, soil microbial biomass, and soil enzymatic activities in wetlands on the southeastern shore of Neuchâtel lake, Switzerland, a lake whose level is partly regulated. Aboveground and belowground plant biomass and correspondent C, N and P concentrations remained constant or decreased moving from the vegetation community subjected to more frequent flooding events to the community with almost no flooding. The soil organic layer exhibited always higher nutrient concentrations and greater enzymatic activities than the organo-mineral and mineral layers. The chemical and biological characteristics of the soil organic layer showed decreasing values for most of the parameters along the hydrological gradient from lakeshore to upland wetland communities. On the basis of nutrient stoichiometry, plant-soil system in the plant community with most flooding events had no-nutrient limitation, while there was a N limitation in the transitional community. In the upland plant community where there was no flooding effect, the plant-soil system was characterized by N and P co-limitation. These findings are important because they provide a threshold for flooding regime by the lake in the context of optimization of lake level regulation under various stakeholders needs.

Keywords: wetland; flooding; plant biomass; biogeochemistry; nutrient stoichiometry

1. Introduction

Wetlands are important ecosystems supporting biodiversity by providing habitats for numerous plants and animals (Neckles et al., 1990; Gibbs, 2000). Furthermore, they ensure several key ecosystem services such as flood control, groundwater replenishment, water purification, carbon (C) sequestration and they provide livelihoods for local populations (Ramsar, 2013). Extreme climate events such as flooding and drought, as well as anthropogenic activities such as sand mining and agriculture intensification (Lai et al., 2014; Xu et al., 2019) are likely to change the hydrological conditions of wetlands (Hingray et al., 2007; Sarneel et al., 2019) and their habitats (Dawson et al., 2003). In particular, variation in frequency and duration of soil flooding and dryness can profoundly affect biogeochemical cycles of C, nitrogen (N) and phosphorous (P) with cascading effects on wetland plant community composition (Baldwin and Mitchell, 2000; Wang et al., 2015b; Swanson et al., 2017).

Flooding regime affects soil organic C and nutrient content and availability by controlling microbial mineralization (Wolf et al., 2013; Wang et al., 2015a; Swanson et al., 2017). Accumulation of degraded organic compounds in water saturated soils and under oxygen deficiency (Hopkinson, 1992; Swanson et al., 2017) leads to increasing dissolved soil organic carbon (DOC) and nutrients, resulting partly from C, N and P leaching from leaf litter under rewetting conditions (Baldwin and Mitchell, 2000; Shrestha et al., 2014). Extracellular degrading enzymes are produced by microorganisms in response to environmental signals or through cell lysis (Sinsabaugh et al., 2009; Cui et al., 2018). It has been proved that their higher activities in wet conditions can accelerate organic C, N and P mineralization rates by breaking down large organic molecules for microbial consumption (Wilson et al., 2011; Heuck et al., 2015). Soil organic carbon (SOC) and nutrient concentrations were shown to be relatively higher in the wetter as compared to the drier part in both river and lake wetlands (Bai et al., 2005; Wang et al., 2016) and this increase of nutrient pool promoted microbial activity and

75 nutrient cycling (Baldwin and Mitchell, 2000; Heuck et al., 2015). Thus, microbial biomass is
76 both a source and a sink for nutrients and participates to nutrient transformation (Gil-Sotres et
77 al., 2005). However, drying of formerly inundated wetlands may cause a severe C limitation
78 and consequently a decrease in the rate of nutrient cycling because of C being lost (Baldwin
79 and Mitchell, 2000) and microbial die-off (Qiu and McComb, 1995).

80 Hydrological conditions in wetlands can have strong impact on plant-soil interactions (Fu
81 et al., 2018; Wang et al., 2018) through changing both organic matter accumulation and
82 mineralization processes (Wilson et al., 2011; Swanson et al., 2017). Plant C, N, P
83 concentrations and their stoichiometric ratios may reflect nutrient availability from substrate
84 and plant nutrient uptake (Demars and Edwards, 2007; Agren, 2008; Elser et al., 2010),
85 Furthermore, the above- and belowground nutrient content and stoichiometry are tightly linked
86 (Bell et al., 2014) and therefore represent important indicators of ecosystem structure and
87 nutrient cycling (Zechmeister-Boltenstern et al., 2015; Sardans et al., 2017). Plant nutrient
88 content and stoichiometry have been studied to detect tissue nutrient allocation (Wang et al.,
89 2015b; Hu et al., 2018), seasonal nutrient variation (Fu et al., 2018), nutrient limitation (Bedford
90 et al., 1999; Demars and Edwards, 2007) or variation between plant functional types (Wang
91 and Moore, 2014; Hu et al., 2018). For example, Li et al. (2017) and Li et al. (2018b) showed
92 that soil nutrient content and stoichiometry are changing along hydrological gradients, and that
93 plant nutrient content and stoichiometry are regulated rather by flooding duration than by soil
94 nutrient content.

95 Different plant species and their abundances can also cause variation in soil and microbial
96 nutrient stoichiometry (De Graaff et al., 2010; Bell et al., 2014) and modulate ecosystem
97 functions through ecological processes affecting microbial and enzymatic activities (Bever et
98 al., 2010; Kardol et al., 2010). There are ample evidences of the close relationships among plant
99 species and soil stoichiometry (Hobbie et al., 2006; Bell et al., 2014; Wang et al., 2018). Pattern

of nutrient availability varies among plant species (Bedford et al., 1999; Güsewell and Koerselman, 2002) and conversely, plants apply some control over nutrient availability by driving soil microbial and enzymatic activities (Richardson et al., 2009; Sardans and Peñuelas, 2012; Bragazza et al., 2015). Along hydrological gradients, the relationship between plant functional traits and soil properties has been explored in wetlands, revealing potential plant-soil relationships (Liu et al., 2015; Wang et al., 2015b; Hu et al., 2018).

In the present study, we investigated soil physico-chemical characteristics (including C, N, P stock and availability), soil microbial biomass nutrients (C, N, P), soil enzymatic activities, and nutrient content in plant and moss biomass (C, N, P) in three plant communities along a hydrological gradient from lakeshore to upland. Investigations took place at the beginning and at peak of the growing season in a wetland along the southern shore of Lake Neuchâtel, Switzerland. We hypothesized that: (i) soil nutrient content, microbial biomass and enzymatic activities, and plant nutrient content are higher in the flooded areas near the lakeshore as compared to the upland area; (ii) the nutrient stoichiometry of plant and soil components can reveal contrasted ecosystem functioning along the hydrological gradient, with a N and P limitation for plant communities of the upland area, as opposed to no N and P limitation for the plant communities close to the lakeshore subjected to flooding.

2. Materials and methods

2.1 Study site

The study was conducted in the wetlands along the southern shore of Lake Neuchâtel (46°54′28″N, 6°52′02″E) in Switzerland, which is the Ramsar site called "Grande Cariçaie". The climate is defined as pluvial sub-oceanic (Buttler, 1990) with a mean annual temperature of 9.4 °C and mean annual precipitation of 891 mm (MeteoSuisse,

https://www.meteosuisse.admin.ch - Payerne, period 1981-2010). Five main plant communities, respectively dominated by *Molinia caerulea* (L.) Moench, *Schoenus nigricans* L., *Carex panicea* L., *Carex elata* All. and *Phragmites australis* (Ca.) Steud. can be found moving from the upland wetland to the lake shoreline (Buttler et al., 1985; Buttler and Gallandat, 1989; Buttler and Muhlhauser, 1994). These plant communities experience an annual flooding regime depending on the lake water level, which is partly regulated and is on average low in winter, high in spring and intermediate in summer and autumn (https://www.hydrodaten.admin.ch/fr/2149.html). Thus, flooding by the intrusion of the lake into the wetland occurs mainly in spring and early summer time, and relates to the snowmelt in the Alps. Heavy rain can recharge the soil water rapidly in autumn, which translates into superficial flooding's event in the upland wetlands which are not influenced by the lake water (Buttler, 1987).

2.2. Sampling design

Distinct plant communities distributed along an elevation gradient (i.e. transects from lakeshore to upland) were selected, a method frequently used in wetland ecosystem studies (e.g. Li et al., 2017; Fu et al., 2018). The three plant communities dominated by *C. elata*, *C. panicea* and *S. nigricans* were selected for this study because they represent the interface between the vegetation influenced by the lake intrusion into the wetland, e.g. *Caricetum elatae* W. Koch 26, variant with *Phalaris arundinaceae*, the transitional vegetation, e.g. *Caricetum elatae* W. Koch 26, variant with *Carex panicea* and the vegetation not reached by the lake, e.g. *Orchio-Schoenetum nigricantis* Oberd. 57, variant with *Galium palustre* (Buttler and Gallandat, 1989). Along this gradient, which is topographically characterized by a gentle slope with about 30-60 cm increase of soil level towards the upland wetland, soils show a marked hydromorphic feature:

149 histic humaquept (*C. elatae* community), wet typic haplaquoll (*C. panicea* community) and dry
150 typic haplaquoll (*S. nigricans* community) (Buttler and Gobat, 1991).
151 Four transects at least 1 km apart were established from the upland to the shoreline
152 (Supplementary Fig. S1). In each of the three plant communities crossed by these transects, one
153 10 m × 10 m plot was randomly selected, leading to a total of 12 plots (4 transects × 3 plant
154 communities). The different botanical compositions in different plots are given in the
155 Supplementary Table S1. One piezometer was installed in each plot to monitor the soil water
156 level (Supplementary Fig. S2). *Carex elata* community was mostly flooded with decreasing
157 water levels during the study period, *C. panicea* community experienced wet conditions with
158 soil saturated in spring and a subsequent drop of water level below -35 cm in August, and *S.*
159 *nigricans* community, typically never flooded with water level decreasing from -4 to -70 cm
160 during the measurement period. Data loggers (Onset HOBO Water Temp Pro v2) were also
161 installed in the soil at 5 cm depth to monitor the soil temperature (Supplementary Fig. S3).
162 Overall, from March to August, the top-soil temperature increased regularly from 5 °C to more
163 than 20 °C, with the soil in *C. elata* community being always slightly colder than that in *C.*
164 *panicea* and *S. nigricans* communities, which had very similar temperatures.
165
166 ***2.3. Plant and soil collection***
167 Plant and soil samples were collected in all plots at the beginning of the plant growing
168 season (April 2018) and at the peak of plant biomass (July 2018) (Supplementary Fig. S4). In
169 addition to the groundwater level measured with the piezometers, the soil volumetric water
170 content was measured at 0-10 cm depth in each plot and at each sampling time using a portable
171 TDR (FieldScout TDR100, UK).
172 Aboveground vascular plant biomass (AGB), moss biomass (MB) and plant litter (L)
173 were collected in all plots using a 50 cm × 50 cm quadrat in *S. nigricans* and *C. panicea*

communities, and a 100 cm × 100 cm quadrat in *C. elata* community. Vascular plants were clipped at the ground level, while litter and mosses were picked up carefully to avoid soil particles. All the aboveground material was placed in separated bags, transported to the laboratory, and then oven-dried at 65 °C for 4 days. Once oven-dried, this plant material was weighted in order to estimate the dry mass (DM) per square meter for each plot and kept for further analysis.

Three soil cores (5.6 cm diameter × 30 cm depth) per plot were randomly collected (in-between the tussocks for *C. elata* and *S. nigricans* communities) for belowground biomass (BGB - roots and rhizomes) sampling. The same layers as for soil sampling (see below) were used and pooled so as to have a composite sample for each layer. Roots and rhizomes were carefully collected by gentle washing in water. As for the aboveground material, the belowground biomass was oven-dried at 65 °C during 4 days and weighted in order to estimate the dry mass (DM) per square meter for each plot and kept for further analysis.

Three other soil cores (5.6 cm diameter × 30 cm depth) per plot were collected in a similar way for the soil sampling as for the belowground biomass. Based on the soil color and texture, each soil core was divided into three parts: organic layer (OL), organo-mineral layer (OML), and mineral layer (ML) (Supplementary Figs. S4 and S5). The top 5 cm of the organic layers from the three soil cores were collected and pooled in one composite sample. For the organo-mineral layer, the 5 cm immediately below the entire organic layer were taken and pooled. For the mineral layer, the upper 2 cm of this layer was removed to avoid irregular transition and the following 5 cm below were collected, which again were pooled in one composite sample. These samples were placed in polyethylene bags, transported to the laboratory and stored at 4 °C until being further processed.

In order to determine the soil bulk density (BD), a last soil core was sampled and an intact piece (5.6 cm diameter × 5 cm depth) was taken for each soil layer and oven-dried at 105 °C

for 48 h and weighted. Bulk density was calculated by dividing the mass of the oven-dried soil sample by its volume and expressed as $g.cm^{-3}$.

2.4. Plant and moss C, N and P analyses

A subsample of dry plant material was washed and dried again, then ground to a fine powder using a ball mill before chemical analysis. Total organic carbon (C) and total nitrogen (N) concentrations were determined by thermal combustion using an elemental analyzer (CE Instruments model NA2500 Nitrogen Carbon Analyzer) and expressed as percent of dry weight. Total phosphorus (P) concentration was determined by the molybdenum blue method after $HClO_4$-H_2SO_4 digestion using a continuous flow autoanalyser (FlowSys, Systea, Anagni, Italy) and expressed as percent of dry weight. Finally, C:N, C:P and N:P stoichiometric ratios were calculated for aboveground and belowground plant biomass, plant litter and moss biomass (Supplementary Fig. S4).

2.5. Soil chemical analyses

For chemical analyses, any visible coarse plant material in the soil samples was removed by hand-sorting. Soil samples were divided into two subsamples: i) a subsample was oven-dried at 65 °C for 4 days and ground to a fine powder using a ball mill in order to determine total organic C (TOC), N (TN) and P (TP) concentrations, and ii) a fresh subsample was stored at 4 °C in order to determine soil pH, nitrate ($N\text{-}NO_3^-$) and ammonium ($N\text{-}NH_4^+$) concentrations, dissolved organic C (DOC), N (DN) and P (DP) concentrations, soil microbial biomass and soil enzymatic activities. For the determination of soil water content (SWC) and in order to quantify all the measured parameters per g of dry soil weight, 10 g of fresh soil samples for each replicate were oven-dried at 105 °C for 48 h. In addition, these oven-dried samples were burned at 550 °C for 6 h in order to determine the soil organic matter content by loss of ignition (LOI).

TOC, TN and TP were determined for each replicate following the same methods as for plant and moss material and expressed as percent of dry soil weight. Soil pH was measured in 1:5 (w/v) soil water suspension with a portable pH meter (WTW multi 3430, Weilheim, Germany) after stirring the mixture for 2 h. $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ concentrations were determined after extraction of 5 g of fresh soil with 30 ml of 1 M KCl extraction and filtration through 0.45 µm filter using a continuous flow autoanalyzer (SEAL Analytical, Germany) and the results were expressed as $mg.kg^{-1}$ oven dry soil.

Microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP) were measured using the chloroform fumigation extraction method (Brookes et al., 1985). Three pairs of about 5 g of fresh soil (3 g for MBP) were weighed for each replicate. One sample from each pair was stored at 4 °C while the other sample was put in a vacuum desiccator and subjected to chloroform vapor. After 24 h of fumigation in the dark at ambient temperature, the fumigated soil samples and the corresponding ones kept unfumigated in the fridge were extracted with a 25 ml of 0.5 M K_2SO_4 for MBC and MBN and with 40 ml of 0.5 M $NaHCO_3$ for MBP. All solutions were filtered through a 0.45 µm filter before analysis. Organic C and N concentrations in the solutions from both fumigated and unfumigated samples were determined using a TOC/TN analyzer (Shimadzu TOC-V), while P concentration was determined by colorimetry using a spectrophotometer at 890 nm (Olsen et al., 1954). Soil DOC, DN and DP were determined as the concentrations obtained from the unfumigated samples. The soil MBC, MBN and MBP values were estimated as the differences between fumigated and unfumigated samples using an extractability factor of 0.45 for C (Vance et al., 1987), 0.54 for N (Brookes et al., 1985) and 0.40 for P (Brookes et al., 1982) and expressed as $mg.kg^{-1}$ oven dry soil.

In order to measure the soil enzymatic activities, 1 g of fresh soil was mixed with 10 ml of water, stirred for 1 h, and the supernatant was collected after centrifugation. The activities of extracellular hydrolase enzymes were measured by adding 50 µl of 4-methylumbelliferyl-ß-

D-glucoside for the activity of ß-glucosidase (BG), 4-MUF-N-acetyl-ß-D-glucosaminide for the activity of ß-1, 4-N-acetylglucosaminidase (NAG), L-leucine-7-amido-4-methycoumarin hydrochloride for the activity of leucine aminopeptidase (LAP) and 4-MUF-phosphate for the activity of phosphatase (PHO) to 250 µl of the soil extract. After 2 h of incubation, the fluorescence was measured on a microplate reader (BioTek SynergyMX) at 450 nm emission and 330 nm excitation wavelength. To quantify product release and account for quenching effects, a set of standards was prepared using methylumbelliferone (MUF) and 7-amino-4-methylcoumarin (MUC) mixed with soil extract. Enzymatic activities were expressed as µmol of substrate (MUF and MUC) converted per min and per g (dry weight) of soil (Freeman et al., 2004).

The C:N, C:P and N:P stoichiometric ratios were calculated for bulk soil and its dissolved fraction and for microbial biomass.

2.6. Data analysis

Statistical analyses were performed with the R software 3.2.3 (R. Core Team, 2017). When necessary, data were log or square root transformed, and the normality and homoscedasticity of the distribution of residuals of models were visually verified.

We used a linear mixed effects model approach (‘‘nlme’’ package, Pinheiro et al., 2020), followed by Tukey HSD tests for post hoc comparisons, to test the effects of vegetation type (*C. elata*, *C. panicea* or *S. nigricans* community), growing season (beginning or peak of the growing season), and their interactions on plant and moss parameters. To consider the fact that we had three plots per transect, the random part of the model indicated that the plots were nested within transects.

For what concerns the soil physico-chemical, microbial and enzymatic parameters, we used a linear mixed-effects model approach followed by Tukey HSD tests for post hoc

comparisons in order to test the effects of soil layers (organic, organo-mineral or mineral layer), vegetation type (*C. elata*, *C. panicea* or *S. nigricans* community), growing season (beginning or peak of the growing season), and their interactions, specifying soil layers nested into plots nested into transects as random factor.

A redundancy analysis (RDA) was used to link the plant chemical characteristics of the three vegetation types taken at two sampling times (C, N and P contents and their stoichiometry in above- and belowground plant biomass, moss biomass and plant litter) as a response to the soil characteristics (chemical and biological variables – see Supplementary Tables S2 and S3).

Finally, a principal component analysis (PCA) was used to determine the correlations between stoichiometry of C, N and P in the plant material, in the bulk soil and in its dissolved fraction, and in the microbial biomass.

3. Results

3.1 Plant and moss parameters

Litter mass and belowground biomass were between 2.4 and 3.5 times higher in *C. elata* community compared to *C. panicea* and *S. nigricans* communities (Table 1 and 2; Supplementary Table S2), while differences in aboveground and moss biomass among the three plant communities were dependent on the sampling time during the growing season (significant vegetation type × growing season interaction, Table 1). At the beginning of the growing season, aboveground biomass of *C. elata* community was lower than that of *C. panicea* and *S. nigricans* communities, while aboveground biomass of *C. elata* community was higher than that of *C. panicea* at the peak of growing season (Fig. 1a). Concerning moss biomass, there was no difference among plant communities at the beginning of growing season, while moss biomass of *C. elata* community was 5 times higher than that of the two other plant communities at the

peak of growing season (Fig. 1b). When summing all plant and moss materials, the organic matter stock was approximately 2.5-time higher in *C. elata* community (10'063 $g.m^{-2}$) as compared to *C. panicea* (4'037 $g.m^{-2}$) and *S. nigricans* (3'803 $g.m^{-2}$) communities. The aboveground biomass increased more than 8 times during the growing season, while litter mass was 30% lower (Table 2; Supplementary Table S2). Belowground biomass decreased by 32% from the beginning to the peak of biomass period, while moss biomass increased 1.6-times, although these trends were not significant (Tables 1 and 2; Supplementary Table S2).

Carbon content in belowground biomass and litter were higher in *C. elata* community compared to *S. nigricans* community (Table 1; Supplementary Table S2). Nitrogen content in aboveground biomass of *C. panicea* community was similar to that of the *C. elata* community and higher than that of *S. nigricans* community at the beginning of the growing season, while it was similar to that of *S. nigricans* community and lower than that of *C. elata* community at the peak of the growing season (significant vegetation type × growing season interaction, Table 1; Fig. 1c). Phosphorus content in aboveground biomass showed a trend of decrease according to the gradient *C. elata* > *C. panicea* > *S. nigricans*, i.e. from lakeshore to upland communities (Table 1; Supplementary Table S2). The decrease in P content in aboveground biomass during the growing season was dependent on the plant community (significant vegetation type × growing season interaction, Table 1), since the P content decreased only in the *C. elata* and C. *panicea* communities (Fig. 1d).

Nitrogen and P content in moss biomass was also higher in *C. elata* compared to *S. nigricans* community (Table 1; Supplementary Table S2). In litter, C content increased, while N content decreased during the growing season, and in belowground biomass both N and P contents decreased during the growing season (Tables 1 and 2; Supplementary Table S2).

3.2 Soil physico-chemical parameters

Bulk density and pH increased according to the gradient organic > organo-mineral > mineral layers (i.e. soil depth) while, in the opposite, all other physico-chemical parameters decreased according to soil depth (Tables 3 and 4; Supplementary Table S3). Moreover, bulk density and pH increased from lakeshore to upland plant communities while, in the opposite, all other physico-chemical parameters decreased according to this vegetation gradient (Tables 3 and 4; Supplementary Table S3). Overall, more physico-chemical parameters were significantly different between *C. elata* and *C. panicea* communities than between *C. panicea* and *S. nigricans* communities (Supplementary Table S3).

Except for soil pH and nitrate, the differences among the three plant communities were dependent on the soil layer (significant soil layer × vegetation type interaction, Table 3), with decreasing differences among plant communities according to increasing soil depth. For example, while we observed marked differences for soil total P (TP) and available P (DP) in organic layer among plant communities, the values of mineral layer were much less marked or similar between plant communities (Fig. 2a and b). The differences in soil available C (DOC) and nitrate contents among the 3 plant communities were also dependent on the growing season (significant vegetation type × growing season interaction, Table 3). Soil DOC values were similar between the 3 plant communities at the beginning of the growing season, while DOC was higher in *C. elata* compared to the two other communities at the peak of growing season (Fig. 3a). Soil nitrate was higher in *C. elata* and *C. panicea* communities compared to *S. nigricans* community at the beginning of the growing season, while similar values between the three plant communities were observed at the peak of growing season (Fig. 3b).

Only few soil physico-chemical parameters varied across the growing season (Table 3). Soil pH and DN increased while, on the opposite, nitrate decreased between the beginning and the peak of the growing season (Tables 3 and 4; Supplementary Table S3). The increase in soil available N (DN) across the growing season was higher in the organic and organo-mineral

layers compared to the mineral layer (significant soil layer × growing season interaction, Table 3; Fig. 4a), while ammonium content increased only in the organic layer (significant soil layer × growing season interaction, Table 3; Fig. 4b).

3.3 Soil microbial biomass and enzyme activity

Soil microbial biomass and enzymatic parameters decreased according to soil depth and according to the vegetation gradient from lakeshore to upland (Tables 3 and 4; Supplementary Table S3). Contrary to microbial biomass, the four enzymatic activities varied across the growing season (Table 3), with higher values reported at the peak compared to the beginning of growing season (Table 4; Supplementary Table S3).

Except for leucine aminopeptidase activity, the differences in microbial biomass and enzymatic parameters among the three plant communities were dependent on the soil layer considered (significant soil layer × vegetation type interaction, Table 3), with decreasing differences according to increasing soil depth (Fig. 2c and d). Finally, the differences in enzymatic parameters among plant communities or among soil layers were stronger at the peak compared to the beginning of growing season (significant soil layers × growing season and vegetation type × growing season interactions, Table 3).

3.4 Plant-soil interactions

The redundancy analysis model (RDA) provides a synthetic view on the relationship between plant chemical characteristics as a response to soil characteristics in the upper organic layer at two sampling periods, April and July (Supplementary Fig. S6). *C. elata* community samples are strongly linked, along axis 1, to high values of N and P in aboveground biomass, moss biomass and plant litter, while belowground biomass samples vary mostly along axis 2, with higher P and N values in April.

The relationships among stoichiometry of C, N and P in all sampled compartments (plant, moss, soil and microbial biomass) in April and July are given in the principal component analysis (PCA) of the Fig. 5. *C. elata* community samples are mostly positively correlated to soil dissolved C:N ratio and strongly negatively correlated to C:P and N:P in microbial biomass. Samples of *C. panicea* community are mostly positively correlated to C:N ratios in belowground biomass, in bulk soil and also weakly in microbial biomass, and strongly negatively correlated to C:P and N:P ratios in bulk soil and in belowground biomass, as well as in soil dissolved C:P. Finally, samples of *S. nigricans* community are positively correlated to several ratios, in particular to C:P and N:P ratios in microbial biomass, to soil dissolved N:P, to bulk soil C:N ratio and to C:N, C:P and N:P ratios in aboveground biomass, moss and litter.

4. Discussion

4.1 Plant biomass and its nutrients content reflect the hydrological gradient induced by the lake flooding

The seasonal pattern of aboveground biomass was different among the three vegetation types. In spring, before the vegetation started to grow, *C. elata* had the lowest aboveground biomass, but later in the season, at the peak of biomass period, the trend reversed and *C. elata* community developed more biomass compared to *C. panicea* and *S. nigricans* communities (in this later case the difference was only marginal). This reflects in the litter accumulation, which was higher in *C. elata* community. Mosses also developed more during the vegetation period in *C. elata* community due to the very favorable microclimatic conditions resulting from high soil moisture and shading conditions under the tussocks. The total plant biomass reflects well the hydrological gradient, with highest values in *C. elata* community and lowest in *S. nigricans* community. Li et al. (2017) found opposite results for biomass distribution along an elevation

399 gradient in Dongting Lake wetland (China), with increasing aboveground biomass with
400 decreasing soil water content. This can be explained by the different hydrological conditions in
401 these two wetlands. In Dongting Lake wetland, the upland plant community still experiences
402 an annual flooding event, similarly to the community close to the lake shoreline and therefore
403 its ecological functioning cannot be assimilated to a conservative nutrient poor biogeochemical
404 cycle as in the *S. nigricans* community investigated in the present study.

405 As we hypothesized, plant nutrient content generally decreased along the hydrological
406 gradient, with higher values in *C. elata* community, which is under the influence of the lake
407 flooding. This was particularly true for N and P content in plant aboveground and moss
408 biomasses. The concentrations of these two elements in aboveground biomass also showed the
409 intermediate status of *C. panicea* community, as exemplified by its N content which was similar
410 to *C. elata* in spring, when both vegetation types were flooded by the lake, and similar to *S.*
411 *nigricans* later in the dryer season (Fig. 1c). Nutrients provided by the lake water are important
412 sources for wetland plants, and indeed, in *C. elata* community, and to some extend also in *C.*
413 *panicea* community, vascular plants and mosses could absorb more nutrients than in *S.*
414 *nigricans* community, which contributed to the higher biomass accumulation. Furthermore, C
415 and nutrients from the litter leachates (Demars and Edwards, 2007; Shrestha et al., 2014) and
416 organic matter accumulation (Wilson et al., 2011; Swanson et al., 2017) under flooding
417 conditions are also beneficial for the plant nutrient absorption and growth.

418 We assessed the nutrient content in aboveground and moss biomass by pooling the plant
419 species, since each plant community was dominated by only a few species (Buttler, 1987, see
420 also Supplementary Table S1). Nevertheless, plant nutrients can show a high interspecific
421 variability and low phenotypic response to nutrient supply (Demars and Edwards, 2007; Li et
422 al., 2017; Hu et al., 2018). Plants have lower nutrient resorption proficiency in nutrient-rich
423 environment (Hopkinson, 1992; Mao et al., 2016), while under extremely low nutrient

availability, they can adapt by maintaining small nutrient concentrations in photosynthetically active tissues (Wang and Moore, 2014). In *S. nigricans* community, we can speculate that plants could transfer and store more nutrients into the living roots. It was also observed that shoots remain partly green over winter, which can contribute to the storage of nutrients, while all the aboveground biomass dies out in *C. elata* vegetation (*personal observations*). As a consequence, in *S. nigricans* community these nutrients could be mobilized during the growing season and allow for relatively high aboveground biomass production as compared to *C. elata* community (Fig. 1a, no statistical difference between CE and SN at the peak of biomass). Nutrient translocation was the reason why litter quality was increased with nutrient enrichment in N-limited wetlands (Mao et al., 2016). We did not detect any vegetation type × growing season cross effect for accumulated litter and its quality (Table 1), so that we assume that the existing litter became similar in the different vegetation types during winter decomposition already. Litter decomposition was mostly related to leaf N content (de Neiff et al., 2006) and flooding can accelerate decomposition through increasing soil moisture (Shrestha et al., 2014; Heuck et al., 2015) and nutrient leaching (Baldwin and Mitchell, 2000; Shrestha et al., 2014). This could explain why, despite initial nutrient concentration differences in early senescent biomass and distinct nutrient translocation capacity in the three communities, there was no significant differences in litter N and P contents. However, C content in litter decreased along the hydrological gradient, with highest values in *C. elata* and lowest in *S. nigricans* communities. This difference of litter C content potentially provides more energy for microorganisms in the *C. elata* community.

From a stoichiometric perspective, in aboveground and moss biomass, C:N, C:P and N:P ratios were significantly lower in *C. elata* and higher in *S. nigricans* communities (Supplementary Table S4). Even if this trend is still visible in the litter, differences were non-significant, which could advocate for similar decomposition rates. In litter bags decomposition

449 experiments, Buttler (1987) found that *k* decomposition rates were 0.229, 0.224 and 0.253 for
450 *C. elata*, *C. panicea* and *S. nigricans* communities, respectively, thus a higher decomposability
451 for the litter in *S. nigricans* community was measured. This discrepancy can be explained by
452 the quality of the litter used. Because of the plant morphology, fresh litter can easily be collected
453 on *S. nigricans* tussocks at the end of the growing season as standing senescent leaves, while
454 for *Carex* species, these senescent leaves tend to fall down and mix with older leaves on the
455 ground, so that litter samples might be more heterogeneous and comprise leaves in a more
456 advanced decomposition stage.
457
458 ***4.2 Soil organic matter quantity and quality reflects the flooding regimes***
459 Under intensive flooding, as happened in *C. elata* community near the lakeshore, there
460 was more than double the amount of organic matter accumulated in the ecosystem, as compared
461 to *C. panicea* and *S. nigricans* communities. With respect to the belowground biomass, which
462 was also highest in *C. elata* community, it cannot be concluded on higher root growth as it was
463 not possible to distinguish between living and dead roots, but lower soil bulk density can
464 indicate higher organic matter content in relation to higher root productivity (Rokosch et al.,
465 2009). As for litter, the strong decrease of belowground biomass during the growing season
466 points to the degradation of dead organic matter. In the belowground biomass, there was no
467 difference in plant N and P concentrations among the three communities, despite different soil
468 nutrient conditions (Table 4). Like for the N:P ratio of above-ground biomass and mosses, the
469 N:P ratio of belowground biomass was higher in the *S. nigricans* community where there is no
470 flooding than in the other two communities. This result is contrary to the finding in Wang et al.
471 (2018) where N:P ratio of belowground biomass was increased with flooding intensity. These
472 opposed results might be explained by distinct nutrient limitation in the two study areas. Below-
473 ground biomass C content decreased along the hydrological gradient, with highest values in *C.*

elata and lowest in *S. nigricans* communities, which mirrors in the trend for higher below-ground biomass C:N and C:P ratios in *C. elata* community. This reflected the humus types, which were marked differently by hydromorphic features: peaty anmoor, anmoor-hydromull and hydromull in *C. elata*, *C. panicea* and *S. nigricans* communities, respectively (Buttler and Gobat, 1991). This is consistent with the decrease of soil organic matter and C contents along the vegetation gradient, as well as in the increase of bulk density and pH (Table 4 and Supplementary Table S3).

4.3 *High organic matter in wetter soils triggers enzyme activity, which in turn accelerates nutrient mineralization*

The soils in the three plant communities differed in their chemical and biological characteristics of their surface organic layer, which showed, as hypothesized, decreasing values for most of the parameters along the hydrological gradient, from *C. elata* to *S. nigricans* communities. Thus, higher soil fertility near the lakeshore also allowed higher microbial biomass. Along the growing season, DOC increased markedly in *C. elata* community, as a result of intense organic matter decomposition of the accumulated litter under high soil biological activity. Thus, higher organic matter contributed to the enzyme activity, which in turn could accelerate nutrient mineralization (Wilson et al., 2011; Heuck et al., 2015). Conversely, nitrate was higher at the beginning of the vegetation period, but decreased thereafter because it was used readily for the high biomass production, and consequently the soils could not be differentiated anymore by their nitrate content at the peak of biomass. Enzyme activity was affected not only by the availability of organic matter, but was also promoted by temperature increase during the growing season (Sinsabaugh et al., 2008; Manzoni et al., 2012).

4.4 Plant-soil interaction and nutrient limitation in different hydrological conditions

The three studied vegetation types are characterized by different nutrient requirements and, according to our hypothesis, this translates into different nutrient limitation and stoichiometric ratios in the plant material, soil and microbial pools. In *C. elata* community, N and P are not limiting because of the regular input of nutrients by lake water. With respect to NO_3, values were on a yearly average 1.7 $mg.L^{-1}$ in the lake water, as opposed to 0.1 $mg.L^{-1}$ in the soil solution of the organic layer in the considered soils, while values for PO_4 were indifferently 0.1 $mg.L^{-1}$ in both the lake water and the upper horizon of these soils, but undetectable in the deeper horizons (Buttler, 1992). This shows that despite the inflow of nutrients from the lake in the wettest plant communities, the nutrient content in the soil does not discriminate the plant communities, despite obvious differences in biomass production and associated nutrient content (this study and Buttler, 1992). This is explained by the immediate uptake of nutrients for biomass production and its feedback effect onto the soil during the vegetation period in wetlands (Dykyjová and Úlehlová, 1978; Bayley et al., 1985). Indeed, hydrological differences among the various vegetation communities can affect soil organic matter accumulation and mineralization processes (Wilson et al., 2011; Swanson et al., 2017), as well as plant nutrient resorption (Wang et al., 2015a). Finally, losses of nutrients from soil organic matter and litter decomposition are important for the eutrophic vegetation influenced by the lake and contribute to a high C:N ratio in the soil dissolved fraction. It has been reported that dissolved organic carbon was higher under flooding than non-flooding condition in floodplain vegetation (Shrestha et al., 2014) and it was increased by flooding duration (Blodau and Moore, 2003; Kim et al., 2014). Therefore, for vegetation under the influence of lake inundation and high nutrient inputs, there is a rapid turnover of nutrients. Conversely, in the upland wetland vegetation characterized by *S. nigicans*, which is never affected by lake flooding, N and P are limiting, which translates in high N:P ratios in the soil dissolved fraction

524 as well as high C:N, C:P and N:P ratios in aboveground biomass, mosses and litter, as well as
525 high C:P and N:P ratios in the microbial biomass. Under such low nutrient availability, nutrient
526 translocation from senescing tissues is a strategy for plants to retard nutrient loss (Hopkinson,
527 1992; Aldous, 2002). This translocation process of nutrients to roots by species such as *S.*
528 *nigricans* (also *Molinia caerulea* and *M. arundinacea, both present in S. nigricans community*)
529 is specific to a conservative biogeochemical cycle. In *C. panicea* community, which has an
530 intermediate position along the vegetation gradient, only N is limiting, which translates into
531 high C:N ratios in bulk soil, below-ground biomass and microbial biomass, and low C:P and
532 N:P ratios in bulk soil and below-ground biomass, as well as low C:P ratio in the soil dissolved
533 fraction.
534
535 **5. Conclusion**
536 High nutrient concentrations in plant leaves tend to be associated with the "live-fast/die
537 young" end of the leaf economics spectrum (Wright et al., 2004; Kazakou et al., 2007), a
538 characteristic which holds for the lakeshore vegetation under the influence of the lake flooding,
539 as opposed to the upland wetland vegetation, which is never reached by lake flooding (Bueche
540 et al., 1994). Our findings have strong implication for wetland management. The wetland
541 hydrodynamic depends strongly on the lake level regulation at the outlet of the lake Neuchâtel,
542 where a dam has been built (Buttler et al., 1995). It is a request to the hydraulic managers to
543 consider the various stakeholders needs for setting the water level curve along the year.
544 Constraints are given by the electricity power plants on the Aar river, navigation on the Rhin
545 river, agriculture in the floodplains around the lake, fisheries and nature conservancy on the
546 south shore of lake Neuchâtel which has become a Ramsar site. In this respect, the wetland
547 vegetation and their soils are sensitive to lake flooding regime, as it was shown in this study. It
548 is therefore important that the water level of the lake is set to optimize the flooding regime in

549 the wetland, more specifically that most of the vegetation of the type of *C. elata* along the
550 lakeshore (but also the *Phragmites communis* belt near the lakeshore and ponds) can be flooded
551 in spring, and the least possible vegetation of the type of *S. nigricans* (and neighboring *Molinia*
552 *coearulea* vegetation) is reached by lake water (Buttler et al., 1995). These findings are
553 important because they provide a threshold for flooding regime by the lake in the context of
554 optimization of lake level regulation under various stakeholders needs. An inadequate water
555 management would affect soil sustainability with a loss of C from the highly organic soils if
556 they would be less flooded. In the opposite, dryer soils would also trigger shrub encroachments
557 and lead to a loss of the most valuable habitats in connection with the lake, which are crucial
558 for many organisms, as for example for fish and bird reproduction. Finally, a general flooding
559 would suppress in the upland wetlands some plant and animal species of high naturalistic
560 importance such as orchids or tree frog.
561
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569
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571
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Tables

Table 1 Effects of vegetation type, growing season, and their interaction on plant and moss parameters. *F*-values and associated *P*-values (* for $P < 0.05$, ** for $P < 0.01$ and *** for $P < 0.001$) are indicated (see values of variables in Supplementary Table S2).

	Vegetation type		Growing season		VT × GS	
Aboveground biomass (AGB)	3.5		189.0	***	13.4	**
C content of AGB	3.5		2.4		0.0	
N content of AGB	17.1	**	0.7		4.5	*
P content of AGB	111.7	***	119.9	***	27.4	***
Litter (L)	14.6	**	7.4	*	1.9	
C content of L	10.0	*	18.0	**	0.4	
N content of L	1.2		9.5	*	0.2	
P content of L	4.5		4.9		2.0	
Moss biomass (MB)	3.2		2.9		7.8	*
C content of MB	3.0		0.6		0.6	
N content of MB	6.5	*	0.8		1.1	
P content of MB	13.2	**	4.7		2.1	
Belowground biomass (BGB)	10.8	**	4.4		0.7	
C content of BGB	7.9	*	4.9		0.1	
N content of BGB	1.9		12.1	**	3.0	
P content of BGB	0.4		32.9	***	1.3	

Table 2 Synthesis of the effects of vegetation gradient and growing season on plant and moss parameters. Vegetation gradient is considered from lakeshore to upland (*i.e.* from *C. elata* to *S. nigricans* community) while growing season is considered from beginning to peak of growing season. ↘ indicates a significant decrease of the value (↘→ indicates a significant decrease only between two vegetation types), ↗ indicates a significant increase of the value and the absence of arrow indicates the absence of a significant effect (see Table 1).

	Vegetation gradient	Growing season
Aboveground biomass (AGB)		↗
C content of AGB		
N content of AGB	↘	
P content of AGB	↘	↘
Litter (L)	↘→	↘
C content of L	↘	↗
N content of L		↘
P content of L		
Moss biomass (MB)		
C content of MB		
N content of MB	↘	
P content of MB	↘	
Belowground biomass (BGB)	↘	
C content of BGB	↘	
N content of BGB		↘
P content of BGB		↘

Table 3 Effects of soil layer, vegetation type, growing season, and their interaction on soil physico-chemical, microbial and enzymatic parameters. *F*-values and associated *P*-values (* for $P < 0.05$, ** for $P < 0.01$ and *** for $P < 0.001$) are indicated (see values of variables in Supplementary Table S3).

	Soil layer		Vegetation type		Growing season		SL × VT		SL × GS		VT × GS		SL × VT × GS	
Bulk density (BD)	243.2	***	21.5	**	1.9		6.2	***	0.3		1.7		0.6	***
Soil water content (SWC)	171.2	***	15.4	**	3.7		2.6	*	0.9		3.1		0.1	
Soil pH	139.8	***	18.4	**	145.3	***	1.0		0.5		2.8		1.3	
Soil organic matter (LOI)	423.1	***	23.4	**	0.6		16.1	***	0.2		1.7		0.2	
Soil organic C (TOC)	160.8	***	15.7	**	0.9		7.0	***	0.3		1.2		0.4	
Soil N (TN)	422.8	***	30.0	***	0.3		11.2	***	0.7		1.9		0.6	
Soil P (TP)	240.3	***	13.5	**	1.3		18.4	***	0.2		0.3		0.6	
Soil available C (DOC)	402.0	***	10.7	**	0.0		10.6	***	1.8		4.8	*	0.5	
Soil available N (DN)	139.8	***	2.7		138.3	***	5.2	**	3.9	*	3.1		1.8	
Soil available P (DP)	372.6	***	19.2	**	2.0		6.5	***	0.2		2.9		1.1	
Ammonium (N-NH4)	193.5	***	7.2	*	0.6		4.6	**	6.0	**	0.0		0.5	
Nitrate (N-NO3)	41.4	***	17.7	**	40.3	***	2.6		2.1		3.2	*	0.5	
Microbial biomass C (MBC)	467.0	***	6.0	*	4.1		14.6	***	0.9		2.9		1.9	
Microbial biomass N (MBN)	804.2	***	11.1	**	4.1		11.5	***	0.5		1.0		0.6	
Microbial biomass P (MBP)	525.9	***	47.4	***	0.3		22.2	***	0.1		0.7		0.6	
ß-glucosidase (BG))	206.9	***	67.5	***	13.6	**	4.4	**	2.7		8.1	**	4.1	**
Leucine aminopeptidase (LAP)	642.1	***	16.0	***	20.5	***	0.6		19.2	***	18.2	***	0.7	
ß -1, 4-N-acetylglucosaminidase (NAG)	156.0	***	54.3	***	29.4	***	12.0	***	2.0		0.7		10.0	***
Phosphatase (PHO)	169.7	***	29.9	***	21.8	***	7.2	***	21.6	***	6.9	**	4.5	**

Table 4 Synthesis of the effects of soil depth increase, vegetation gradient and growing season, on soil physico-chemical, microbial and enzymatic parameters. Soil depth increase is considered from surface organic to lower mineral layer, vegetation gradient from lakeshore to upland (*i.e.* from *C. elata* to *S. nigricans* community), while growing season is considered from beginning to peak of growing season. ↘ indicates a significant decrease of the value (↘→ indicates a significant decrease only between two vegetation types), ↗ indicates a significant increase of the value and the absence of arrow indicates the absence of a significant effect (see Table 3).

	Increasing soil depth	Vegetation gradient	Growing season
Soil physico-chemical parameters			
Bulk density (BD)	↗	↗	
Soil water content (SWC)	↘	↘	
Soil pH	↗	↗	↗
Soil organic matter (LOI)	↘	↘	
Soil organic C (TOC)	↘	↘	
Soil N (TN)	↘	↘	
Soil P (TP)	↘	↘	
Soil available C (DOC)	↘	↘→	
Soil available N (DN)	↘		↗
Soil available P (DP)	↘	↘	
Ammonium (N-NH4)	↘	↘→	
Nitrate (N-NO3)	↘	↘	↘
Microbial parameters			
Microbial biomass C (MBC)	↘	↘	
Microbial biomass N (MBN)	↘	↘	
Microbial biomass P (MBP)	↘	↘	
Enzymatic parameters			
ß-glucosidase (BG)	↘	↘	↗
Leucine aminopeptidase (LAP)	↘	↘	↗
ß -1, 4-N-acetylglucosaminidase (NAG)	↘	↘	↗
Phosphatase (PHO)	↘	↘	↗

806 **Figure legend**

807

808 **Fig. 1** Aboveground plant biomass (a), moss biomass (b), N content in aboveground plant
809 biomass (c) and P content in aboveground plant biomass (d) according to the vegetation type
810 × growing season interaction (Table 1). Each bar represents the mean value ± SE; n= 4. For
811 (a), (b) and (c), different letters denote significant differences among plant communities
812 according to the selected growing season period with a > b > c. For (d), stars indicate significant
813 differences between the beginning and the peak of growing season according to the selected
814 plant community with * for $P < 0.05$, ** for $P < 0.01$ and *ns* for $P > 0.05$. CE = *C. elata*, CP =
815 *C. panicea*, SN = *S. nigricans*, GS = growing season.

816

817 **Fig. 2** Soil total P content (a), available P content (b), microbial biomass C (c) and leucine
818 aminopeptidase activity (d) according to the soil layer × vegetation type interaction (Table 3).
819 Each bar represents the mean value ± SE; n= 8. Different letters denote significant differences
820 among plant communities according to the selected soil layer with a > b > c. CE = *C. elata*, CP
821 = *C. panicea*, SN = *S. nigricans*. LAP = leucine aminopeptidase.

822

823 **Fig. 3** Soil available C (a) and nitrate (b) contents according to the vegetation type × growing
824 season interaction (Table 3). Each bar represents the mean value ± SE; n= 12. Different letters
825 denote significant differences among vegetation types according to the selected growing season
826 period with a > b. CE = *C. elata*, CP = *C. panicea*, SN = *S. nigricans*.

827

828 **Fig. 4** Soil available N (a) and ammonium (b) content according to the soil layer × growing
829 season interaction (Table 3). Each bar represents the mean value ± SE; n= 12. Stars indicate
830 significant differences between the beginning and the peak of growing season according to the
831 selected soil layer with * for $P < 0.05$, ** for $P < 0.01$, * ** for $P < 0.001$ and *ns* for $P > 0.05$.
832 GS = growing season.

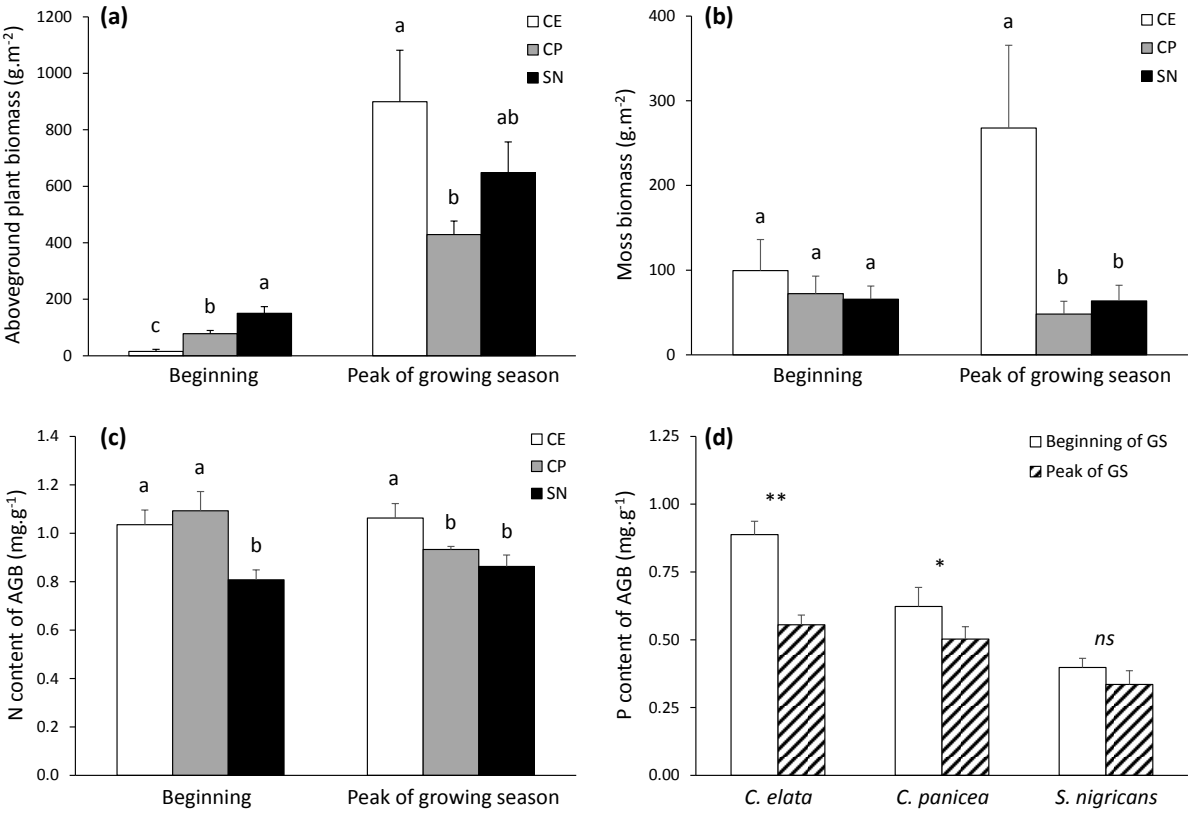
833

834 **Fig. 5** Principal component analysis (PCA) illustrating the relationship between stoichiometry
835 of C, N and P in the plant material (above and belowground biomass, moss and litter), in the
836 bulk soil and in its dissolved fraction, and in the microbial biomass in the organic layer at the
837 two sampling periods, April and July. Samples are labelled with sampling period (A: April, J:
838 July) and vegetation type (CE: *Carex elata*, CP: *Carex panicea*, SN: *Schoenus nigricans*).
839 Variables are labelled as in Tables 1-4.

840

841 **Fig. 1**

842

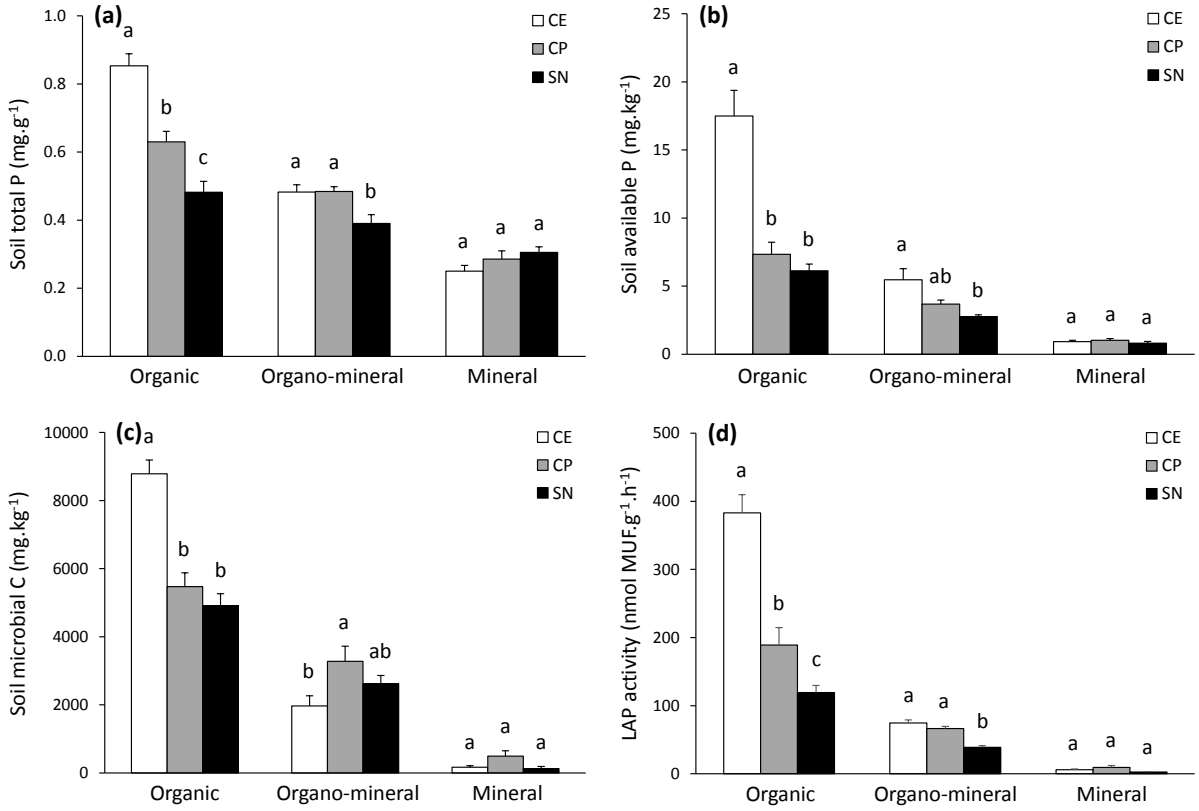


843

844

845 **Fig. 2**

846

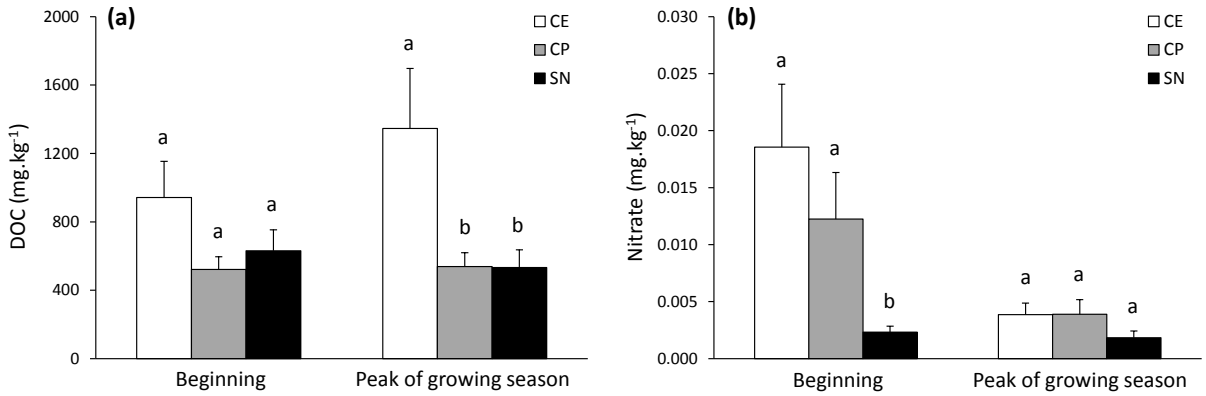


847

848

849 **Fig. 3**

850



851

852

853 **Fig. 4**

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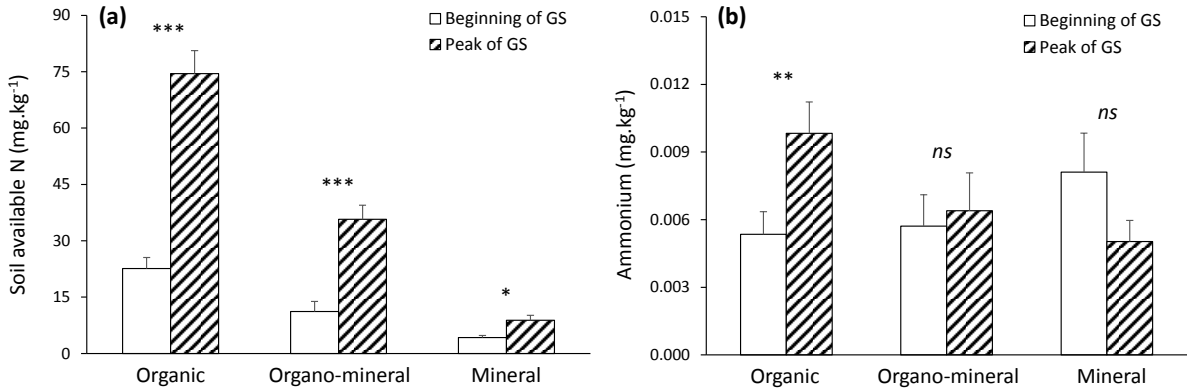
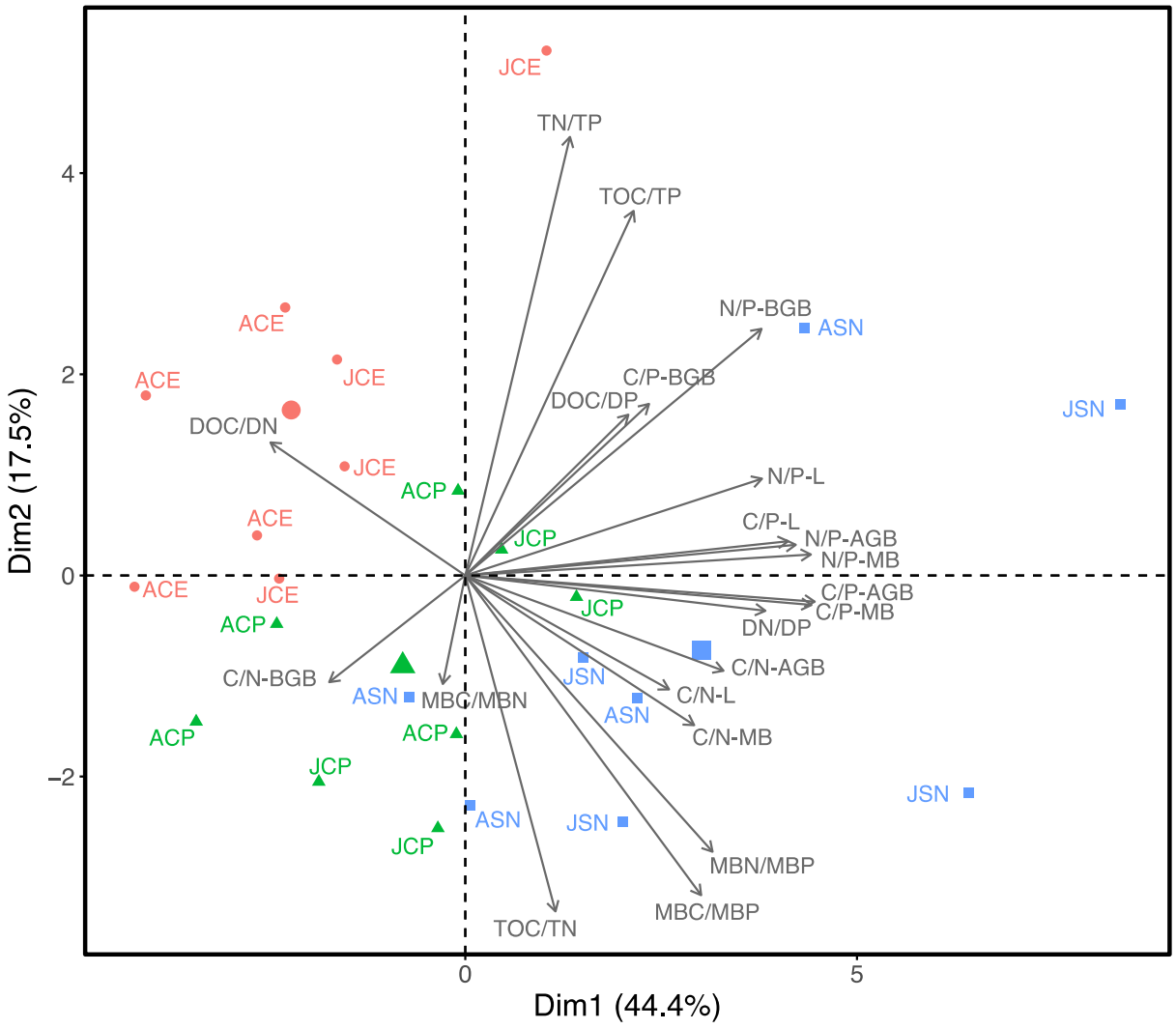


855

856

857 **Fig. 5**

858



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