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ORIGINAL PAPER

Litter N:P ratios indicate whether N or P limits the decomposability of graminoid leaf litter

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Abstract The N:P ratio of leaf litter may determine if decomposability is N-limited (litter with low N:P ratio) or P-limited (litter with high N:P ratio). To test this hypothesis and to determine the threshold between N and P limitation, we studied relationships between litter N and P concentrations, litter mass loss and effects of fertilisation on litter mass loss in laboratory experiments. Leaf litter of 11 graminoid species was collected in Swiss and Dutch wetlands, yielding 84 litter samples with a broad range of N and P concentrations (3.2–15.1 mg N g^{-1}, 0.04–1.93 mg P g^{-1}) and with N:P mass ratios ranging from 5 to 100. On nutrient-free sand, dry mass loss after five or ten weeks (5.5–53% of initial mass) correlated positively with the N and P concentrations of the litter. Within species, mass loss correlated mainly with N for litter with low N:P ratio, and with P for litter with high N:P ratio, in agreement with our hypothesis. Among species, however, these relationships did not exist, and decomposition rather correlated with the specific leaf area. When the litter was incubated on fertilised sand, 35 out of 50 litter samples decomposed faster than on nutrient-free sand. Decomposition was generally accelerated by P fertilisation (i.e. P-limited) when the N:P ratio of the litter was above 25 and the P concentration below 0.22 mg g^{-1}, supporting our hypothesis. N-limited decomposition was not significantly related to the litter N:P ratio but occurred rarely for litter with N:P ratio greater than 25, and only for litter with N concentration below 11.3 mg g^{-1}. We conclude that the N:P ratio of leaf litter indicates whether its decomposability is more likely to be N- or P-limited. The critical N:P ratio (threshold between N and P limitation) appeared to be 25 for graminoid leaf litter.

Keywords Decomposition · Ecological stoichiometry · Laboratory incubation · Nutrient limitation · Phosphorus · Wetland plants

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Introduction

N:P ratios of plant biomass have been widely used as indicators of nutrient limitation for primary production (Güsewell 2004; Koerselman and Meuleman 1996; Tessier and Raynal 2003). They also determine whether the supply of N and P to herbivores, pathogens or decomposers is

proportional to the needs of these consumers (Elser et al. 2000a; Moe et al. 2005; Smith 2002). If the N:P ratio of plant biomass or litter differs from that required by consumers, the latter will experience a relative shortage or N or P, which may reduce their growth, the rate of food consumption and the efficiency with which carbon as well as the non-limiting nutrient are used (Hessen et al., 2004; Raubenheimer and Simpson, 2004). In particular, N:P ratios of plant litter may determine whether N or P limits the rate of litter mass loss and whether N or P is released more rapidly by the decomposing litter (Hobbie and Vitousek 2000).

Although the decomposition of terrestrial plant litter is often limited by low carbon quality (Ågren et al. 2001; Bridgham and Richardson 2003), fertilisation experiments have shown that decomposition can also be nutrient-limited (Allison and Vitousek 2004; Hobbie and Vitousek 2000; Rejmánková 2001). Nutrient limitation is most commonly found with lignin-poor, rapidly decomposing litter during the early stage of decomposition (Knorr et al. 2005). In this case, litter N:P ratios may indicate which of the two elements is more limiting, and the question arises whether a certain 'critical N:P ratio' discriminates between N- and P-limited decomposition, similar to the 'critical N:P ratios' for biomass production (Koerselman and Meuleman 1996).

Several 'critical N:P ratios for decomposition' have been suggested in the literature. In a meta-analysis of published decomposition rates, Aerts (1997) found that the decomposition of temperate litter with an average N:P ratio of 11.9 correlated more with its N concentration than with its P concentration. In contrast, the decomposition of tropical litter with an average N:P ratio of 16.0 correlated more with its P concentration. Aerts (1997) concluded that a litter N:P ratio of 10–15 discriminates between N- and P-limited decomposition. In a more recent review, Smith (2002) proposed a critical N:P ratio of 9 based on the fact that decomposition rates correlated with the P concentration for litter with N:P > 9 but not for litter with N:P < 9. Using plant material from a growth experiment, Güsewell and Freeman (2005) found that decomposition was always P-limited for litter with N:P ratio above 22, but could be N- or P-limited for litter with lower N:P ratios.

Inconsistent 'critical N:P ratios' for decomposition may be due to differing ways of assessing nutrient limitation. Some studies compared the decomposition rates of litter samples with contrasting nutrient concentrations, either across or within species (Cornelissen et al. 2000; Vitousek 1998; Xu and Hirata 2005), while others examined how the decomposition of particular litter samples responds to fertilisation (Allison and Vitousek 2004; Rejmánková 2001) or considered the effects of both internal (litter) and external (fertiliser) nutrients (Bridgham and Richardson 2003; Hobbie and Vitousek 2000). Furthermore, some studies considered decomposition at ecosystem level, comparing litter produced and decomposed in differently fertilised plots (Aerts et al. 2006; Aerts et al. 2001), while others focused on litter decomposability, i.e. decomposition under standardised and favourable conditions, except for variation in nutrient supply (Cornelissen et al. 2000; Güsewell and Freeman 2005). The second approach is most likely to reveal relationships between litter quality and the nutrient limitation of decomposers, though implications for natural decomposition processes remain to be investigated.

Here we tested whether N:P ratios determine if N or P limits the decomposability of leaf litter from field-grown wetland graminoids. We focused on this functional plant group to reduce the influence of non-nutritional factors such as tissue structure or secondary chemistry (Cornelissen et al. 1999). The litter of wetland graminoids typically has a low lignin content (< 10%, Aerts and de Caluwe 1997a; Aerts et al. 2003) and low nutrient concentrations, which are the conditions under which decomposition is most likely to be nutrient-limited (Knorr et al. 2005).

We investigated both the role of 'internal' litter nutrients and the effects of 'external' nutrient addition by incubating litter with a wide range of N and P concentrations (inter- and intraspecific variation) on N- and P-free sand as well as with various nutrient additions in a laboratory experiment. We expected that on nutrient-free sand, decomposition would correlate mainly with the P concentration of the litter for litter samples with

high N:P ratios, and mainly with the N concentration for litter samples with low N:P ratios. We further expected that P addition would accelerate the decomposition of litter samples with high N:P ratios, whereas N addition would accelerate the decomposition of litter samples with low N:P ratios.

Materials and methods

To analyse relationships between litter nutrients and decomposition across a large number of litter samples with widely differing nutrient concentrations and N:P ratios, we combined the data of two experiments, using litter collected in Swiss and Dutch wetlands, respectively.

Collection and decomposition of Swiss litter

The Swiss sites are fen meadows near Zürich (430–550 m a.s.l.; mean annual temperature: 9.4°C, mean annual precipitation: 1,144 mm) on base-rich gleysols. The vegetation is of *Molinion* or *Filipendulion* type, with a peak above-ground biomass of 300–800 g m^{-2} in August (Güsewell and Klötzli 1998). Sites are mown in September or October. Litter of four sedge and two grass species (Table 1) was sampled at two to seven sites by collecting the youngest fully senesced leaf of 20–50 shoots at the end of August 2000, just before mowing. Litter from a certain species at a certain site will hereafter be called a 'litter sample'; there were 28 litter samples from Switzerland (Table 1).

After collection, the litter was cut into pieces of 0.5–1 cm length and air-dried in paper envelopes. The decomposition rate of each litter sample was determined through laboratory incubations derived from the comparative method of Wardle et al. (1998) and respiration assays of R. Aerts and colleagues (Aerts and de Caluwe 1997a; Aerts et al. 2003). Approximately 150 mg of air-dried litter was weighed to the nearest 0.1 mg and placed in Petri dishes (6 cm diameter, with aeration slits) above 18 ± 0.5 g of quartz sand covered with a disk of 0.3-mm polyethylene mesh (Monodur, Verseidag-Techfab, Geldern, Germany). Sand and litter were then wetted with 8 ml of soil surface water (pH 7.0) collected in a fen co-dominated by *Carex acutiformis* and *Phragmites australis* to inoculate them with a natural decomposer community. A small addition of detergent to the water (0.1 g l^{-1} sodium dodecyl sulphate) facilitated the wetting of the litter after air-drying (cf. Taylor 1998). According to preliminary experiments, the detergent did not affect the rate of decomposition once the litter was moist (S. Güsewell, unpublished data).

Table 1 Plant species included in the decomposition experiment with the number of litter samples (*n*), litter N and P concentrations and N:P mass ratios (means), specific leaf area (data from Clevering 1998; Güsewell 1997, 2005) and percentage litter mass loss in 5 weeks (Switzerland, CH) or 10 weeks (The Netherlands, NL). The nomenclature follows Tutin et al. (1964–1993)

Sites	Species	*n*	N conc. (mg g^{-1})	P conc. (mg g^{-1})	N:P ratio	SLA (cm^2 g^{-1})	% mass loss	
							mean	range
CH	*Carex acutiformis*	4	5.4	0.14	42.1	186.7	11.1	7.5–13.2
	Carex elata	4	6.4	0.13	62.6	219.5	9.8	5.5–13.0
	Carex flacca	2	4.7	0.17	28.0	185.0	9.9	9.2–10.6
	Carex flava	3	6.9	0.26	26.9	254.2	8.0	6.5–09.3
	Carex panicea	4	4.6	0.12	43.0	189.8	12.0	8.6–17.6
	Molinia caerulea	4	4.0	0.07	60.8	286.3	13.0	7.0–15.9
	Phragmites australis	7	7.1	0.22	40.6	190–210[a]	15.5	12.4–21.1
NL	*Anthoxanthum odoratum*	13	12.2	1.47	8.5	314.8	43.0	36.6–53.0
	Carex acutiformis	13	9.9	0.56	19.2	101.1	21.6	16.6–34.9
	Carex curta	17	8.3	0.95	11.5	225.4	26.7	19.7–37.0
	Carex diandra	4	8.5	1.04	12.6	n.d.	23.3	20.5–26.8
	Eriophorum angustifolium	9	6.9	0.34	26.8	118.8	17.1	9.3–30.0

[a]Range of values for the low-N treatment in Clevering (1998)

The effects of nutrient addition on decomposition were tested by adding 7.5 mg N as NH_4NO_3 or 0.3 mg P as KH_2PO_4 (dissolved in the soil surface water) to some Petri dishes. These nutrient additions were tenfold the mean amounts of N and P contained in the litter subsamples and did not influence the pH of the water in the Petri dish, which was 6.2–6.6 at the end of the incubation.

Petri dishes were placed in cardboard boxes and kept in an incubator at 22°C and 50% air moisture. The decomposing material was sprinkled with deionised water after the first week of incubation and thereafter every 2 weeks to replace water lost through evaporation (about 2 ml per Petri dish) and to simulate leaching that would be associated with rainfall in the field. After 5 weeks, the litter was picked from the mesh with tweezers and dried for 48 h at 75°C to determine final dry mass. Due to the limited amount of litter available, there were only 1–4 replicates per litter sample and nutrient treatment, and some litter samples were incubated only in the control treatment (without nutrient addition).

Collection and decomposition of Dutch litter

The Dutch sites are three floating fens on former peat cuttings in the region of Utrecht (0 m a.s.l.; mean annual temperature: 9.0°C, mean annual precipitation: 732 mm). A thick floating mat of roots, rhizomes and *Sphagnum* mosses with pH 4–6 supports vegetation of *Caricion davallianae* or *Caricion nigrae* type with an above-ground biomass of 200–700 g m^{-2} in July (Verhoeven et al. 1996; 1988; Verhoeven and Schmitz 1991). Sites are mown annually in July or August. To obtain litter with a wide range of nutrient concentrations, we sampled plots from a fertilisation experiment (Güsewell et al. 2002, 2003). At each site, 16 plots of 50 × 50 cm^2 were fertilised with N (20 g m^{-2}), P (5 g m^{-2}), both elements (NP-treatment), or none (control), in 1999 and 2000. All plots also received 16 g m^{-2} of K. Nutrients were applied as diluted solutions of NH_4NO_3, NaH_2PO_4 and KCl. To investigate the effects of fertiliser on vegetation biomass (the original purpose of the experiment), the above-ground biomass of the central 40 × 40 cm^2 of each plot was clipped in July 2000 (Güsewell et al. 2002).

To obtain litter for our decomposition study, the border zone of the plots (which had been fertilised but not clipped in July) was clipped in November 2000, when plants had largely senesced. The 2–3 most recently senesced leaves per shoot were sampled for the most abundant species, i.e. the grasses *Anthoxanthum odoratum* and *Calamagrostis canescens* and the sedges *Carex acutiformis, C. curta, C. diandra*, and *Eriophorum angustifolium*. Due to the small size of the sampling area, only a few shoots of the aforementioned species were present in each individual plot, and many samples were smaller than the minimum of 300 mg litter required for nutrient analyses and incubations. In total, 56 litter samples could be obtained for the present study (Table 1). In the analysis and presentation of results, we shall not include the fertiliser treatments explicitly, which would be problematic because the heterogeneous distribution of plant species among plots created an incomplete design. The effect of treatments on decomposition will be considered implicitly by relating decomposition to litter N and P concentrations; it was also tested in simplified, two-way Anovas (factor treatment and species, ignoring sites) but results will be only briefly mentioned.

The decomposition experiment was carried out with the same procedures as for the Swiss litter, except for the following differences (1) the pH of the soil surface water used to wet the litter (from a floating fen co-dominated by *Carex acutiformis* and *Phragmites australis*) was 6.0, i.e. slightly lower (2) the amounts of N and P added in the nutrient treatments were higher (11.4 mg N and/or 0.96 mg P per Petri dish) because the Dutch litter was more nutrient-rich; we verified in separate incubations with a range of nutrient additions that the resulting nutrient concentrations caused no toxic effects (3) a combined N + P treatment was also included to test for co-limitation by N and P (4) incubations lasted for 10 weeks.

Control experiments

To verifiy that the experimental procedure was suitable to detect nutrient limitation, cellulose as a nutrient-free, labile source of carbon was incubated for 10 weeks with the two types of soil

surface water and with the four nutrient treatments. The cellulose lost 40–70% of its initial mass with the addition of N + P but only 10% in the three other treatments (Fig. 1). This pronounced difference shows that the soil surface water was an effective microbial inoculum but not a source of either N or P to the microbes, since both elements had to be added to obtain rapid decomposition. Thus, microbial growth in the litter decomposition experiments would entirely depend on the N and P contents of the litter, plus the N or P added in the nutrient treatments. There was no difference between the water types used in the Swiss and Dutch experiments, respectively (Fig. 1).

To further verifiy that results from the two experiments were comparable, the *P. australis* litter from the seven Swiss sites was also incubated with the ''Dutch'' procedure. Both results are shown in graphs with distinct symbols for visual comparison, but only the 5-week incubations were used in statistical data analysis.

Analyses of litter nutrient concentrations

Subsamples of 150–200 mg litter were dried at 75°C for 48 h to determine the water content. These subsamples were then digested with a modified Kjeldahl method (1 h at 200°C and 2 h at 340°C in a mixture of concentrated sulphuric acid, salicylic acid, copper and selenium) and analysed colorimetrically for total N and P concentrations on a continuous-flow analyser (Skalar SA–40, Skalar, Breda, NL).

The carbon content of the litter was determined for a few samples on a C/N analyser (Perkin Elmer 2400 Series II) and proved to be nearly constant (mean ± SD = 46.3% ± 1.3%), so that variation in C/N and C/P ratios would be determined by variation in N and P concentrations, respectively. The lignin content was also determined for a few samples (*P. australis*, *C. acutiformis*, *C. elata*) using the method of Poorter and Villar (1997); it ranged from 4 to 5%, similar to values reported elsewhere for graminoid leaf litter (Aerts and de Caluwe 1997a; Aerts et al. 2003).

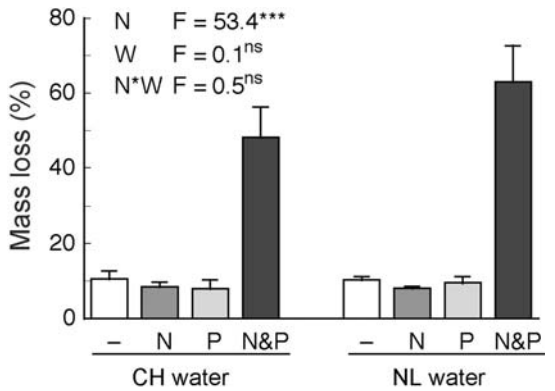

Fig. 1 Percentage of dry mass lost by cellulose during 10 weeks of decomposition in Petri dishes at 22°C with four nutrient treatments (combinations of adding or not adding mineral N and P to the sand; ''–'' = unfertilised control treatment), after inoculation with the two water types used to incubate the Swiss and Dutch litter, respectively. Bars show means + *SE* (n = 4). *F*-ratios and the significance of the effects of nutrient addition (N), water type (W) and the interaction (N*W) were determined with two-way Anova

Data analysis

Litter mass loss during the incubation was calculated for each Petri dish as percentage of initial dry mass. Data from the Swiss and Dutch experiments were first analysed separately. For a joint analysis, mass loss after 5 weeks (Swiss litter) was converted into an estimate of mass loss after 10 weeks using a calibration established from 18 litter samples (Fig. 2).

To examine how decomposition depends on internal nutrient concentrations, we considered only the results from incubations without nutrient

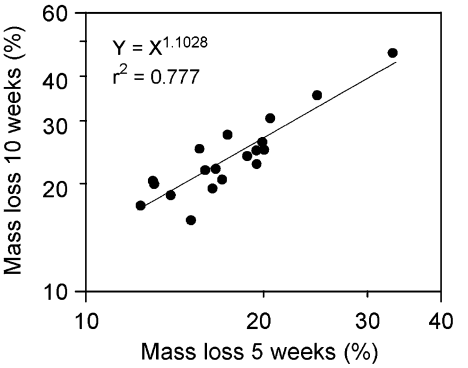

Fig. 2 Relationship between the percentage of dry mass lost by leaf litter during 5 and 10 weeks of decomposition in Petri dishes, based on 18 litter samples from five species and used to compare data from the experiments with Swiss and Dutch litter in this study. The species (and numbers of litter samples) were *P. australis* (7), *C. acutiformis* (4), *C. elata* (5), *C. flava* (1), *M. caerulea* (1)

addition and calculated pairwise correlations (Pearson's *r*) between litter mass loss and log-transformed N and P concentrations or N:P ratios of the litter. These correlations were calculated at three levels (1) *overall correlations*, across all litter samples (2) *interspecific correlations*, across species means (3) *intraspecific correlations*, across individual litter samples after adjusting for species means.

The effects of external nutrient addition on decomposition were calculated for each litter sample and for each nutrient treatment (N, P, N + P) as the difference between the (mean) % dry mass loss with nutrient addition and the (mean) % dry mass loss without nutrient addition. For many litter samples, these differences could not be tested statistically as there were only 1–2 replicates per treatment. We therefore determined a threshold for ''significant'' treatment effects as follows: For the 12 litter samples with 3–4 replicates in all treatments, one-way ANOVAs showed that the least significant ($\alpha = 0.05$) difference in % mass loss between treatments ranged from 3.6 to 9.1%, with an average of 5.98% (for 10-week incubations). A difference of 5.98% between nutrient treatments was therefore regarded as ''significant'' in all cases. This criterion was chosen to minimise both type I errors (find 'effects' due to chance) and type II errors (fail to detect real effects), as both error types could distort relationships between the occurrence of treatment effects and litter quality (Stefano 2003).

Based on the responses to external nutrient addition, the decomposition of each litter sample was classified as N-limited, P-limited, co-limited by N and P (stimulated by the joint addition of N and P but not by either nutrient alone), and not limited by N or P. The occurrence of each type of nutrient limitation was related to litter N and P concentrations and N:P ratios with second-order logistic regression. If the quadratic term was clearly not significant ($P > 0.25$), a first-order model was fitted.

Results

Across the 84 litter samples, N concentrations varied fivefold, litter P concentrations fiftyfold, and N:P ratios twentyfold (Fig. 3). Litter from Dutch sites was on average more nutrient-rich than litter from Swiss sites. Litter N and P concentrations correlated positively with each other, especially when compared among species (Fig. 3, Table 2). Because P concentrations varied more than N concentrations (overall $CV = 88.7\%$ for P vs. 33.6% for N), litter N:P ratios decreased with increasing N and P concentrations (Fig. 3).

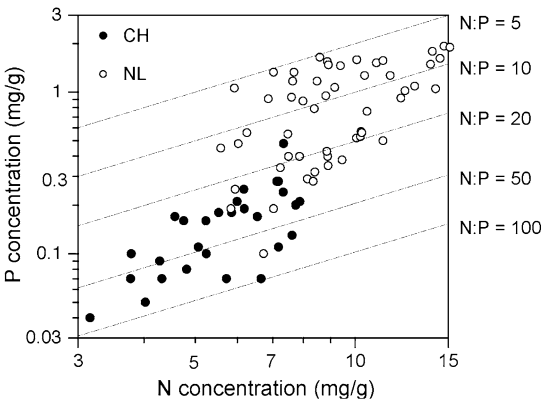

Fig. 3 Relationships between the N and P concentrations and N:P ratios of all litter samples. Closed symbols: swiss litter, open symbols: Dutch litter; both axes with logarithmic scale

Litter mass loss during incubations without nutrient addition ranged from 5.5 to 21.1% after 5 weeks and from 9.3 to 53.0% after 10 weeks (Fig. 4). This variation resulted from two- to threefold variation among species as well as within species (Table 1). Litter mass loss correlated positively with N and P concentrations (Fig. 4a,b) and negatively with N:P ratios (Fig. 4c). These correlations were significant across the entire data set and across the Dutch litter samples but not across the Swiss litter samples (Table 2). Interspecific correlations between litter mass loss and nutrient concentrations were positive for the Dutch litter but not significant with the Swiss litter (Table 2). Intraspecific correlations were positive in both cases, but litter mass loss correlated best with P concentrations for the Swiss litter, whereas it correlated best with N concentrations for the Dutch litter (Table 2).

Nutrient addition during the incubation increased or decreased litter mass loss. Based on mass loss after 10 weeks (using estimated values for the Swiss litter), the effect of N addition

Table 2 Inter- and intraspecific correlations (Pearson's *r*) between N and P concentrations of the litter (N–P) as well as between litter nutrient concentrations (N, P or N:P ratios) and litter mass loss (MLoss). Interspecific correlations were calculated from the means of the *n* species from Swiss (CH) and Dutch (NL) sites, respectively. Intraspecific correlations were calculated from *n* litter samples after adjusting for species means. All calculations used log-transformed nutrient concentrations

		n	N–P	N–MLoss	P–MLoss	N:P–Mloss
Overall	CH	28	0.71^{***}	0.17	0.29	–0.28
	NL	53	0.56^{***}	0.74^{***}	0.71^{***}	-0.56^{***}
Interspecific	CH	7	0.77^{*}	–0.10	–0.29	0.40
	NL	5	0.80	0.85	0.88	–0.76
Intraspecific	CH	28	0.52^{**}	0.26	0.61^{***}	-0.64^{***}
	NL	53	0.24	0.61^{***}	0.42^{**}	–0.20

The significance of correlations is indicated as $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; no symbol, $P > 0.1$.

ranged from –15.4 to + 15.3% of initial mass (mean: + 1.7%), the effect of P addition ranged from –12.3% to + 17.9% (mean: + 1.0%), and the effect of N + P addition ranged from –14.0 to + 37.6% (mean: + 5.2%).

N-limited decomposition was found for litter with intermediate N concentration (5.9–11.3 mg N g^{-1}; Fig. 5a). It was not significantly related to the litter P concentration (Fig. 5b) or N:P ratio, although N limitation occurred rarely for litter with N:P ratio greater than 25 (Fig. 5c). Litter with P-limited decomposition had on average lower N and P concentrations and higher N:P ratios than the other litter samples (Fig. 5); therefore, the probability of decomposition being P-limited was negatively related to the litter N and P concentrations (Fig. 5a,b) and positively related to the litter N:P ratio (Fig. 5c). Co-limitation by N and P (only studied for the Dutch litter) was not significantly related to the litter nutrients (Fig. 5).

Discussion

Intra- versus inter-specific correlations as indicators of nutrient limitation

In our laboratory incubations, litter mass loss correlated positively with the N and P concentrations

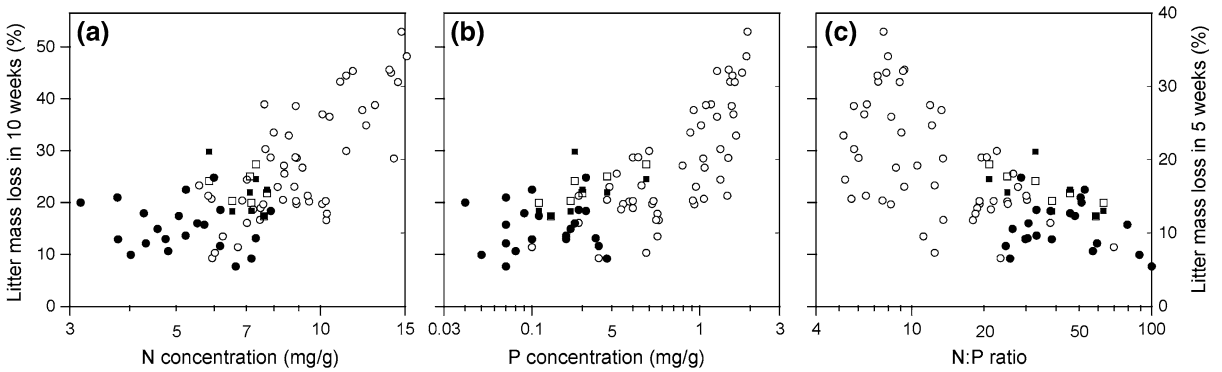

Fig. 4 Percentage of dry mass lost by leaf litter during 5 or 10 weeks of decomposition in Petri dishes on N- and P-free sand (control treatment) in relation to (**a**) the N concentration (**b**) the P concentration, and (**c**) the N:P ratio of the litter. Symbols show means per litter sample; squares represent litter of *Phragmites australis*, which was incubated during both 5 and 10 weeks; circles represent all other species (closed circles: Swiss litter, open circles: Dutch litter). The scale of the x-axis (nutrient concentrations) is logarithmic; the y-axis is scaled differently for 5-week and 10-week incubations based on the relationship in Fig. 2

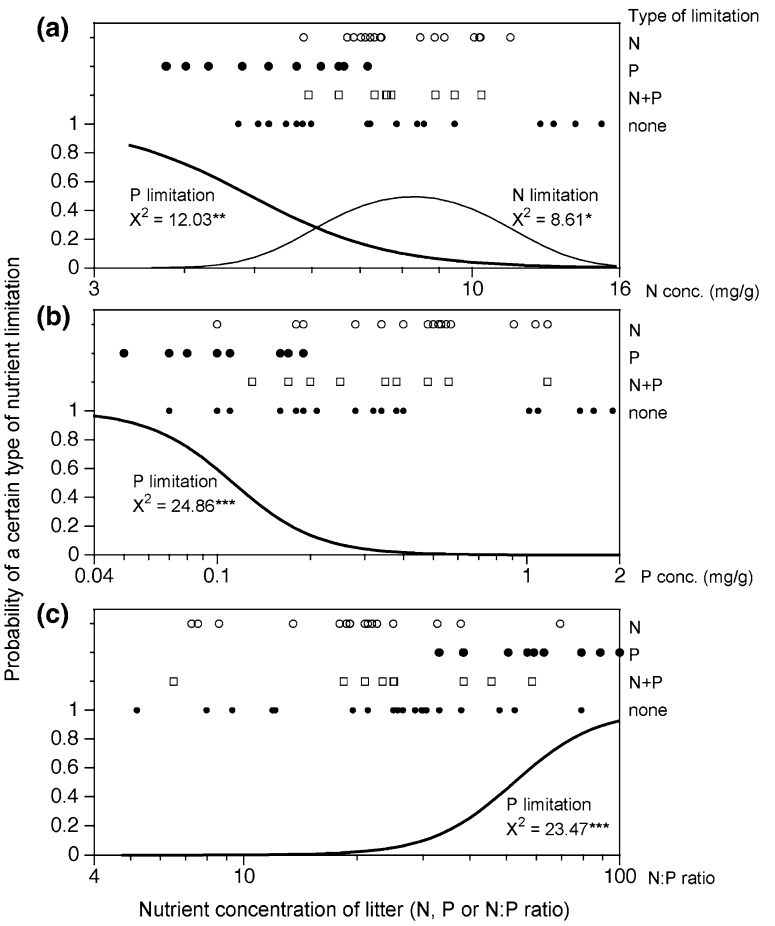

Fig. 5 Relationships between (**a**) the N concentration (**b**) the P concentration and (**c**) the N:P ratio of leaf litter (log scale) and the nutrient limitation of litter decomposition. In the upper part of the graphs, each symbol represents one litter sample and shows which nutrient treatment accelerated its decomposition. In the lower part of the graphs, curves represent the probability of decomposition being limited by a certain element, as modelled with first- or second-order logistic regression, together with Chi^2 values and significance levels from likelihood ratio tests; only significant relationships are shown

of the litter, suggesting that decomposition was generally limited by the internal availability of N and P. However, the N and P concentrations correlated with each other, so that their effects on decomposition were confounded. This difficulty has been encountered in all studies comparing litter samples across a broad range of N and P concentrations (Coulson and Butterfield 1978; Enriquez et al. 1993; Thompson et al. 1997). A strong correlation between N and P concentrations of litter typically results from a positive correlation between N and P concentrations of plant biomass as well as between N and P resorption efficiency during leaf senescence (Aerts 1996; Güsewell 2005). These correlations are usually more pronounced among species than within species (Table 2; see also Güsewell 2004). Accordingly, the effects of N and P on litter mass loss are less confounded and can better be compared on the basis of intraspecific correlations than on the basis of interspecific correlations.

Intraspecific correlations

Within species, mass loss mainly correlated with P concentrations for the Swiss litter, and with N concentrations for the Dutch litter. Given that the Swiss litter had higher N:P ratios than the Dutch litter, these results are consistent with the hypothesis that litter N:P ratios determine whether internal N or P plays a greater role for decomposition (Aerts 1997; Smith 2002). At the Dutch sites, intraspecific variation in P concentration (but not in N concentration) was largely due to the previous fertilisation of the plots (Güsewell et al. 2002). Yet, litter mass loss was unrelated to fertiliser treatments (data not

shown). This further indicates that litter P concentrations did not limit decomposition, consistent with the low N:P ratios.

Interspecific correlations

Among the seven Swiss species, litter mass loss correlated negatively (though not significant) with litter P concentrations, in contrast to the positive correlation found within species. Interspecific differences in decomposition are often related to structural and carbon-related parameters of litter quality (Aerts and de Caluwe 1997b; Allison and Vitousek 2004; Hobbie 1996). Although we studied only graminoids to reduce the role of these non-nutritional parameters, they may still have influenced litter decomposition: for 17 graminoid species, Cornelissen and Thompson (1997) found that decomposition rates were not only related to the N, P and K concentrations of their leaves but also to their specific leaf area (SLA), leaf life span and leaf toughness. The seven Swiss species all had a similar SLA (Table 1) and accordingly, similar rates of decomposition. Among the five Dutch species, the species decomposing most rapidly (*A. odoratum*) had a threefold greater SLA than the two species with slowest decomposition (*C. acutiformis*, *E. angustifolium*), and *C. curta* was intermediate (Table 1). Thus, interspecific differences in tissue structure (as reflected by the specific leaf area) may have contributed to the observed interspecific differences in decomposition rates. This confounding is almost inevitable because of the overall association between structural and nutritional traits in leaves of vascular plants (Nielsen et al. 1996; Reich et al. 1999; Wright et al. 2004). Even when intraspecific differences in litter nutrient concentrations are created through fertilisation, the confounding is not necessarily avoided because fertilisation can affect structural properties of the litter, e.g. by reducing the lignin content (Vitousek 1998).

We conclude that correlations between internal concentrations of N or P and decomposition rates do not indicate reliably which nutrient limits decomposition; nutrient addition experiments are usually preferable. If such experiments are not possible, intraspecific correlations are better indicators of nutrient limitation than interspecific correlations.

Effects of external nutrient addition

Incubations in Petri dishes with nutrient addition confirmed our hypothesis that decomposition could be stimulated by the addition of either N or P (relative to a nutrient-free control treatment), depending on the litter N:P ratio. Decomposition was P-limited only for litter with N:P ratio above 25. This N:P ratio is higher than the range of N:P ratios typically found in the soil microbial biomass (1–10 according to Anderson and Domsch 1980; Brookes et al. 1984; Williams and Silcock 2001). A possible reason is that most of the litter P is in the form of inorganic phosphates or P esters (Chapin and Kedrowski 1983). Ester-bonded P is readily hydrolysed by the phosphatases of microorganisms and plant roots (Chauhan et al. 1981; Tarafdar and Claassen 1988). As a result, the actual availability of P relative to N during decomposition is probably higher than suggested by the litter N:P ratio (McGill and Cole 1981). Many studies found P to be released more rapidly than N from decomposing litter (Harris et al. 1995; Scheffer and Aerts 2000), except when litter N:P ratios were extremely high (Handreck 1997). In addition, the low N:P ratios mostly reported for soil microbes may reflect luxury uptake of P when energy (labile carbon) limits microbial growth (Schmidt et al. 1997). When microbes are nutrient-limited, their N:P ratios are wider and more variable (Brookes et al. 1984; Tezuka 1990).

Because we analysed data from two separate experiments, it was important to check whether results are comparable despite the methodological differences. The litter of *P. australis* sampled from seven fen meadows (squared symbols in Fig. 3), showed the same type of nutrient limitation with both experimental protocols, except that for two litter samples, decomposition was limited by N or P after 5 weeks but jointly limited by N and P after 10 weeks. Earlier experiments with other graminoid species had also shown similar effects of nutrient addition on litter decomposition after 5 and 10 weeks (Güsewell et al. 2005 and unpublished data).

In our data set, P-limited decomposition not only occurred exclusively at litter N:P ratios above 25 but was also restricted to litter with a P concentration below 0.22 mg g^{-1}. Obviously the P concentration must be sufficiently low for decomposition to be P-limited, regardless of the N:P ratio (Bosatta and Ågren 1991). In agreement with the present results, other experiments with field-grown litter found decomposition to be P-limited when the litter P concentration was below 0.2–0.3 mg g^{-1}. For example, N-limited decomposition was found for litter of *Myriophyllum spicatum* with 0.63 mg P g^{-1} (N:P ratio of 19.4; Carpenter and Adams 1979), but P-limited decomposition for litter from *Quercus rubra* with 0.21 mg P g^{-1} (N:P ratio of 34.3; Elwood et al. 1981). For *Metrosideros polymorpha* in rain forest, decomposition was P-limited for litter with 0.21 mg P g^{-1} but not for litter with 0.51 mg P g^{-1}, although the N:P ratio was 17 in both cases (Hobbie and Vitousek 2000). A P concentration of 0.2–0.3 mg g^{-1} may correspond to the amount of P that is bound in structural compounds (remaining after 'complete resorption' according to Killingbeck 1996) and therefore less readily available to decomposers. With litter from a pot experiment, Güsewell & Freeman (2005) found P-limited decomposition up to a litter P concentration of 0.5 mg g^{-1} but suggested that the artificial experimental conditions might have led to the production of litter with more labile carbon, promoting faster-growing microbes with greater P requirements (Elser et al. 2003; Makino et al. 2003).

The fact that litter P concentrations below 0.3 mg g^{-1} are uncommon under natural conditions (Enriquez et al. 1993) may explain why P is rarely found to limit decomposition in field experiments, sometimes even for litter with N:P ratios well above 20 (Aerts et al. 2003; Aerts et al. 2001; Coulson and Butterfield 1978). Furthermore, decomposing litter with a P concentrations below 0.3 mg g^{-1} tends to immobilise P even from P-poor soils (e.g. Xu and Hirata 2005). Since the decomposition of such P-poor litter is likely to be dominated by slow-growing microbes with low P requirements (Elser et al. 2003; Elser et al. 2000b), the amount of P immobilised from the soil may be sufficient to prevent decomposition from being stimulated by additional P fertilisation (Hobbie and Vitousek 2000). In our Petri dishes, no P could be immobilised from the sand, which was virtually P-free.

The occurrence of N-limited decomposition was not significantly related to the litter N:P ratio, probably because samples with lowest N:P ratios had high N and P concentrations (Fig. 3). However, decomposition was rarely N-limited when the N:P ratio exceeded 25, the threshold above which P-limited decomposition could occurr. Thus, with few exceptions, a litter N:P ratio of 25 did discriminate between N- and P-limited decomposition.

N-limited decomposition was not found for those litter samples which had the lowest N concentrations but for those with intermediate N concentrations. Litter samples with N concentrations below 5 mg g^{-1} also had P concentrations below 0.2 mg g^{-1} (Fig. 3), which caused decomposition to be P-limited, rather than N-limited. On the other hand, decomposition was never N-limited for litter with a N concentration above 11.3 mg g^{-1}. This value was only exceeded by a few litter samples because wetland graminoids generally have low nutrient concentrations in their leaves (Güsewell and Koerselman 2002) and high nutrient resorption efficiency (Aerts 1996). In forbs and deciduous woody species, litter N concentrations above 11.3 mg g^{-1} are more common (Dorrepaal et al. 2005), which may contribute to explaining why their decomposition is more rarely N-limited. An ancillary experiment with ten litter samples from graminoids, forbs and deciduous woody species, with a broad range of N concentrations and with P concentrations above 0.3 mg g^{-1} supported this interpretation: the effect of N fertilisation on decomposition was negatively related to the litter N concentration, and decomposition was stimulated by N only for litter with less than 11 mg N g^{-1} (Fig. 6).

Conclusions

Our study has shown that for leaf litter of wetland graminoids, significant relationships exist between N:P ratios of the litter and whether decomposition

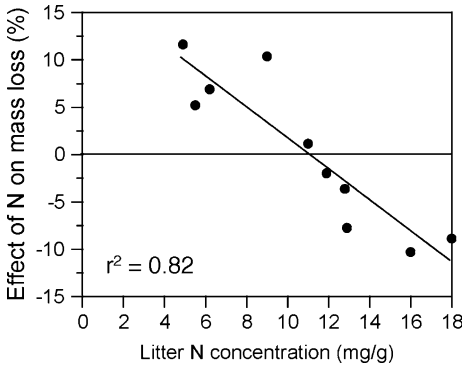

Fig. 6 Relationship between the N concentration of leaf litter and the effect of N fertilisation on its decomposition, as determined in a additional experiment with ten litter samples from graminoids, forbs and deciduous woody species. Methods were as for the main experiment. The effect of N was determined as the difference in % litter mass loss after 10 weeks between fertilised and unfertilised Petri dishes

in a nutrient-free environment is N- or P-limited. Both nutrient addition experiments and intraspecific comparisons of litter samples revealed these relationships, whereas interspecific comparisons did not, probably due to confounded effects. Theory about the decomposition process suggests that relationships between N:P ratios and nutrient limitation of decomposition may depend on the carbon chemistry of the litter and on whether the litter is generally nutrient-rich or nutrient-poor (Sterner and Elser 2002). For graminoid litter, which has a low lignin content and low nutrient concentrations, a 'critical N:P ratio' of 25 appeared to indicate whether decomposition is more likely to be N-limited or P-limited. Additionally, N concentrations below 11.3 mg g^{-1} and P concentrations below 0.3 mg g^{-1} were required for decomposition to be N- or P-limited, respectively. Similar experiments with litter from other functional and taxonomic groups are needed to generalise our results.

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References

Aerts R (1996) Nutrient resorption from senescing leaves of perennials: are there general patterns? J Ecol 84:597–608

Aerts R (1997) Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. Oikos 79:439–449

Aerts R, de Caluwe H (1997a) Initial litter respiration as indicator for long-term leaf litter decomposition of *Carex* species. Oikos 80:353–361

Aerts R, de Caluwe H (1997b) Nutritional and plant-mediated controls on leaf litter decomposition of *Carex* species. Ecology 78:244–260

Aerts R, de Caluwe H, Beltman B (2003) Plant community mediated vs. nutritional controls on litter decomposition rates in grasslands. Ecology 84:3198–3208

Aerts R, van Logtestijn RSP, Karlsson PS (2006) Nitrogen supply differentially affects litter decomposition rates and nitrogen dynamics of sub-arctic bog species. Oecologia 146:652–658

Aerts R, Wallén B, Malmer N, de Caluwe H (2001) Nutritional constraints on *Sphagnum* growth and potential decay in northern peatlands. J Ecol 89:292–299

Ågren GI, Bosatta E, Magill AH (2001) Combining theory and experiment to understand effects of inorganic nitrogen on litter decomposition. Oecologia 128:94–98

Allison SD, Vitousek PM (2004) Extracellular enzyme activities and carbon chemistry as drivers of tropical plant litter decomposition. Biotropica 36:285–296

Anderson JPE, Domsch KH (1980) Quantities of plant nutrients in the microbial biomass of selected soils. Soil Sci 130:211–216

Bosatta E, Ågren GI (1991) Theoretical analysis of carbon and nutrient interactions in soils under energy-limited conditions. Soil Sc Soc Am J 55:728–733

Bridgham SD, Richardson CJ (2003) Endogenous versus exogenous nutrient control over decomposition and mineralization in North Carolina peatlands. Biogeochem 65:151–178

Brookes PC, Powlson DS, Jenkinson DS (1984) Phosphorus in the soil microbial biomass. Soil Biol Biochem 16:169–175

Carpenter SR, Adams MS (1979) Effects of nutrients and temperature on decomposition of *Myriophyllum spicatum* L. in a hard-water eutrophic lake. Limnol Oceanogr 24:520–528

Chapin FSI, Kedrowski RA (1983) Seasonal changes in nitrogen and phosphorus fractions and autumn retranslocation in evergreen and deciduous taiga trees. Ecology 64:376–391

Chauhan BS, Stewart JWB, Paul EA (1981) Effect of labile inorganic phosphate status and organic carbon additions on the microbial uptake of phosphorus in soils. Can J Soil Sci 61:373–385

Clevering OA (1998) An investigation into the effects of nitrogen on growth and morphology of stable and die-back populations of *Phragmites australis*. Aquat Bot 60:11–25

Cornelissen JHC, Perez Harguindeguy N, Diaz S, Grime JP, Marzano B, Cabido M, Vendramini F, Cerabolini B (1999) Leaf structure and defence control litter decomposition rate across species and life forms in regional floras on two continents. New Phytol 143:191–200

Cornelissen JHC, Pérez-Harguindeguy N, Gwynn-Jones D, Díaz S, Callaghan TV, Aerts R (2000) Autumn leaf colours as indicators of decomposition rate in sycamore (*Acer pseudoplatanus*). Plant Soil 225:33–38

Cornelissen JHC, Thompson K (1997) Functional leaf attributes predict litter decomposition rate in herbaceous plants. New Phytol 135:109–114

Coulson JC, Butterfield J (1978) An investigation of the biotic factors determining the rates of plant decomposition on blanket bogs. J Ecol 66:631–650

Dorrepaal E, Cornelissen JHC, Aerts R, Wallen B, van Logtestijn RSP (2005) Are growth forms consistent predictors of leaf litter quality and decomposability across peatlands along a latitudinal gradient? J Ecol 93:817–828

Elser JJ, Acharya K, Kyle M, Cotner J, Makino W, Markow T, Watts T, Hobbie SE, Fagan W, Schade J, Hood J, Sterner RW (2003) Growth rate-stoichiometry couplings in diverse biota. Ecol Lett 6:936–943

Elser JJ, Fagan WF, Denno RF, Dobberfuhl DR, Folarin A, Huberty A, Interland S, Kilham SS, McCauley E, Schulz KL, Siemann EH, Sterner RW (2000a) Nutritional constraints in terrestrial and freshwater food webs. Nature 408:578–580

Elser JJ, Sterner RW, Gorokhova E, Fagan WF, Markow TA, Cotner JB, Harrison JF, Hobbie SE, Odell GM, Weider J (2000b) Biological stoichiometry from genes to ecosystems. Ecol Lett 3:540–550

Elwood j W, Newbold JD, Trimble AF, Stark RW (1981) The limiting role of phosphorus in a woodland stream ecosystem: effects of P enrichment on leaf decomposition and primary producers. Ecology 62:146–158

Enriquez S, Duarte CM, Sand-Jensen K (1993) Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. Oecologia 94:457–471

Güsewell S (1997) Evaluation and management of fen meadows invaded by *Phragmites australis*. Diss. ETH No 12'428, Zürich

Güsewell S (2004) N:P ratios in terrestrial plants: variation and functional significance. New Phytol 164:243–266

Güsewell S (2005) Nutrient resorption of wetland graminoids depends on the type of nutrient limitation. Funct Ecol 19:344–354

Güsewell S, Freeman C (2005) Nutrient limitation and enzyme activities during litter decomposition of nine wetland species in relation to litter N:P ratios. Funct Ecol 19:582–593

Güsewell S, Jewell PL, Edwards PJ (2005) Effects of heterogeneous habitat use by cattle on nutrient availability and litter decomposition in soils of an Alpine pasture. Plant Soil 268:135–149

Güsewell S, Klötzli F (1998) Abundance of common reed (*Phragmites australis*), site conditions and conservation value of fen meadows in Switzerland. Acta Bot Neerl 47:113–129

Güsewell S, Koerselman W (2002) Variation in nitrogen and phosphorus concentrations of wetland plants. Persp Ecol Evol Syst 5:37–61

Güsewell S, Koerselman W, Verhoeven JTA (2002) Time-dependent effects of fertilisation in Dutch floating fens. J Veg Sci 13:705–718

Güsewell S, Koerselman W, Verhoeven JTA (2003) N:P ratios as indicators of nutrient limitation for plant populations in wetlands. Ecol Appl 13:372–384

Handreck KA (1997) Phosphorus requirements of Australian native plants. Aust J Soil Res 35:241–289

Harris TT, Williges KA, Zimba PV (1995) Primary productivity and decomposition of five emergent macrophyte communities in the Lake Okeechobee marsh ecosystem. Archivum Hydrobiologii, Species Issues Advances in Limnology 45:63–78

Hessen DO, Agren GI, Anderson TR, Elser JJ, De Ruiter PC (2004) Carbon sequestration in ecosystems: the role of stoichiometry. Ecology 85:1179–1192

Hobbie SE (1996) Temperature and plant species control over litter decomposition in Alaskan tundra. Ecol Monogr 66:503–522

Hobbie SE, Vitousek PM (2000) Nutrient limitation of decomposition in Hawaiian forests. Ecology 81:1867–1877

Killingbeck KT (1996) Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. Ecology 77:1716–1727

Knorr M, Frey SD, Curtis PS (2005) Nitrogen additions and litter decomposition: a meta-analysis. Ecology 86:3252–3257

Koerselman W, Meuleman AFM (1996) The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. J Appl Ecol 33:1441–1450

Makino W, Cotner JB, Sterner RW, Elser JJ (2003) Are bacteria more like plants or animals? Growth rate and resource dependence of bacterial C:N:P stoichiometry. Funct Ecol 17:121–130

McGill WB, Cole CV (1981) Comparative aspects of cycling of organic C, N, S, and P through soil organic matter. Geoderma 26:267–286

Moe SJ, Stelzer RS, Forman MR, Harpole WS, Daufresne T, Yoshida T (2005) Recent advances in ecological stoichiometry: insights for population and community ecology. Oikos 109:29–39

Nielsen SL, Enríquez S, Duarte CM, Sand-Jensen K (1996) Scaling maximum growth rates across photosynthetic organisms. Funct Ecol 10:167–175

Poorter H, Villar R (1997) The fate of acquired carbon in plants: chemical composition and construction costs. In: Bazzaz FA, Grace J (eds) Plant Resource Allocation. Academic Press, San Diego pp 39–72

Raubenheimer D, Simpson SJ (2004) Organismal stoichiometry: quantifying non-independence among food components. Ecology 85:1203–1216

Reich PB, Ellsworth DS, Walters MB, Vose JM, Gresham C, Volin JC, Bowman WD (1999) Generality of leaf trait relationships: a test across six biomes. Ecology 80:1955–1969

Rejmánková E (2001) Effect of experimental phosphorus enrichment on oligotrophic tropical marshes in Belize, Central America. Plant Soil 236:33–53

Scheffer RA, Aerts R (2000) Root decomposition and soil nutrient and carbon cycling in two temperate fen ecosystems. Oikos 91:541–549

Schmidt IK, Michelsen A, Jonasson S (1997) Effects of labile soil carbon on nutrient partitioning between an arctic graminoid and microbes. Oecologia 112:557–565

Smith VH (2002) Effects of resource supply ratios on the structure and function of microbial communities. Antonie van Leeuwenhoek 81:99–106

Stefano D (2003) How much power is enough? Against the development of an arbitrary convention for statistical power calculations. Funct Ecol 17:707–709

Sterner RW, Elser JJ (2002) Ecological Stoichiometry. Princeton University Press, Princeton, NJ, USA

Tarafdar JC, Claassen N (1988) Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. Biol Fertil Soils 5:308–312

Taylor BR (1998) Air-drying depresses rates of leaf litter decomposition. Soil Biol Biochem 30:403–412

Tessier JT, Raynal DY (2003) Use of nitrogen to phosphorus ratios in plant tissue as an indicator of nutrient limitation and nitrogen saturation. J Appl Ecol 40:523–534

Tezuka Y (1990) Bacterial regeneration of ammonium and phosphate as affected by the carbon:nitrogen:phosphorus ratio of organic substrates. Microb Ecol 19:227–238

Thompson K, Parkinson JA, Band SR, Spencer RE (1997) A comparative study of leaf nutrient concentrations in a regional herbaceous flora. New Phytol 136:679–689

Tutin TG, et al. (eds) (1964–1993) Flora Europaea. Vols 1–5. Cambridge University Press, Cambridge, UK

Verhoeven JTA, Beltman B, de Caluwe H (1996) Changes in plant biomass in fens in the Vechtplassen area, as related to nutrient enrichment. Netherlands J Aquat Ecol 30:227–237

Verhoeven JTA, Koerselman W, Beltman B (1988) The vegetation of fens in relation to their hydrology and nutrient dynamics: a case study. In: Symoens JJ Eds. Vegetation of inland waters. Kluwer Academic Publishers, Dordrecht pp 249–282

Verhoeven JTA, Schmitz MB (1991) Control of plant growth by nitrogen and phosphorus in mesotrophic fens. Biogeochem 12:135–148

Vitousek PM (1998) Foliar and litter nutrient, nutrient resorption, and decomposition on Hawaiian *Metrosideros polymorpha*. Ecosystems 1:401–407

Wardle DA, Barker GM, Bonner KI, Nicholson KS (1998) Can comparative approaches based on plant ecophysiological traits predict the nature of biotic interactions and individual plant species effects in ecosystems? J Ecol 86:405–420

Williams BL, Silcock DJ (2001) Does nitrogen addition to raised bogs influence peat phosphorus pools? Biogeochem 53:307–321

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas M-L, Niinemets U, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R (2004) The worldwide leaf economics spectrum. Nature 428:821–827

Xu X, Hirata E (2005) Decomposition patterns of leaf litter of seven common canopy species in a subtropical forest: N and P dynamics. Plant Soil 273:279–289