

# Research Project : Enzyme activity during N- and P-limited decomposition of wetland plant litter

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## RESEARCH PROJECT

### Enzyme activity during N- and P-limited decomposition of wetland plant litter

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#### Summary

1 The decomposition of plant litter in wetlands is often limited by the availability of either nitrogen (N) or phosphorus (P) in litter or soil. The aim of our research is to test whether the type of nutrient limitation can be predicted from the litter chemistry (N concentration, P concentration, N:P ratio) and/or from the activities of three enzymes during initial decomposition:  $\beta$ -glucosidase (involved in C mineralisation), amidase (N mineralisation) and phosphatase (P mineralisation). We hypothesise that the activity of amidase is high relative to the two other enzymes when decomposition is N-limited, and the activity of phosphatase when decomposition is P-limited.

2 In a preliminary experiment, green and senesced leaves of *Carex elata* were incubated on sand that was fertilised with N or P, or left unfertilised. Enzyme activity was assayed in microbial suspensions obtained from the leaves after five weeks of decomposition. The relative activities of the three enzymes were partly modified by fertilisation: the  $\beta$ -glucosidase/phosphatase activity ratio on senesced leaves was increased by fertilisation with N+P, and the amidase/phosphatase activity ratio on green leaves was higher after P fertilisation than after N fertilisation. This lends support to our hypothesis that enzyme activity ratios reflect nutrient availability to decomposers.

3 For the main experiment plants of nine wetland species (forbs, grasses and sedges) are grown at six N:P supply ratios to obtain 54 leaf litter types with widely varying N and P concentrations. Enzyme activity during the decomposition of each litter type will be determined after 3–6 weeks of incubation on unfertilised sand. Incubations on N- or P-fertilised sand will be carried out to determine which nutrient limits decomposition and to relate the type of limitation to ratios of enzyme activities.

4 The present project deals with the initial stages of decomposition under laboratory conditions. If we find relationships between relative enzyme activities and nutrient limitation, as hypothesised, further research will be needed to assess whether these relationships also hold for the later stages of litter decomposition and under field conditions.

#### Zusammenfassung

##### Enzymaktivität beim Abbau von Pflanzenstreu aus Feuchtgebieten

1 Der Abbau von Pflanzenstreu in Feuchtgebieten kann durch die Verfügbarkeit von Stickstoff (N) oder Phosphor (P) im Pflanzenmaterial bzw. im Boden begrenzt werden.

In diesem Projekt testen wir, ob der begrenzende Nährstoff anhand der Nährstoffkonzentrationen im Pflanzenmaterial oder anhand der Aktivitäten von drei Enzymen ( $\beta$ -Glucosidase, Amidase, Phosphatase) beurteilt werden kann. Wir vermuten, dass die relative Aktivität von Amidase (im Vergleich zu den beiden anderen Enzymen) hoch ist, wenn N den Abbau begrenzt, und dass die relative Aktivität von Phosphatase hoch ist, wenn P den Abbau begrenzt.

2 In einem Vorversuch wurden lebende und tote Blätter von *Carex elata* auf Sand mit oder ohne Zugabe von N und P inkubiert. Die Enzymaktivität wurde nach fünf Wochen in mikrobiellen Suspensionen bestimmt. Die relativen Aktivitäten der drei Enzyme hingen zum Teil von der Nährstoffzugabe ab: Auf den toten Blättern wurde das Verhältnis von  $\beta$ -Glucosidase- zu Phosphatase-Aktivität durch Zugabe von N+P erhöht, und auf den grünen Blättern war das Verhältnis von Amidase- zu Phosphatase-Aktivität bei Zugabe von P grösser als bei Zugabe von N. Dies unterstützt unsere Vermutung, dass relative Enzymaktivitäten die Nährstoffverfügbarkeit widerspiegeln.

3 Für den Hauptversuch werden Pflanzen von neun Arten bei sechs verschiedenen N:P-Verhältnissen kultiviert, um 54 Typen von Blattstreu mit sehr verschiedenen N- und P-Konzentrationen zu erhalten. Die Aktivitäten der drei Enzyme werden nach Inkubation ohne Nährstoffzugabe bestimmt, und zusätzliche Inkubationen mit Zugabe von N oder P werden zeigen, welcher der beiden Nährstoffe für den Abbau von jedem Blatttyp begrenzend ist.

4 Falls die vermuteten Zusammenhänge zwischen Nährstoffbegrenzung des Abbaus und relativen Enzymaktivitäten in diesen kurzfristigen Laborversuchen gefunden werden, bleibt zu untersuchen, ob solche Zusammenhänge auch längerfristig und unter Feldbedingungen gültig sind.

**Keywords:** *Carex* litter; enzyme assays; fertilisation; methylumbelliferyl substrates; nutrient limitation; N:P ratios

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## Introduction

Nutrient availability is one of the main factors regulating the production and the decomposition rates of plant biomass and thus, the carbon balance of ecosystems. Several human impacts currently affect the nutrient availability in temperate wetlands, including atmospheric nitrogen deposition (Morris 1991; Bobbink *et al.* 1998), inflow of nutrient-rich waste water and run-off (Carpenter *et al.* 1998), hydrological changes (Olde Venterink *et al.* 2002), climate warming (Rustad *et al.* 2001), changes in management (Güsewell *et*

*al.* 2000; Güsewell 2003), or plant invasions (Emery & Perry 1996). Besides increasing or reducing nutrient availability in wetlands, these impacts can modify the relative availability of nitrogen (N) and phosphorus (P). This may cause biomass production and litter decomposition to shift from N limitation to P limitation or vice-versa (Aerts *et al.* 1992; Verhoeven *et al.* 1996) and may affect the responses of these processes to future nutrient enrichment (Gordon *et al.* 2001). If production and decomposition respond differently to

nutrient enrichment, carbon storage is altered (Aerts *et al.* 1995; Lamers *et al.* 2000). In ecological models simulating the effects of human perturbations on carbon storage in wetlands, it may be important to account for type of nutrient limitation of the processes involved (Aerts *et al.* 1995). This requires the ability to assess which nutrient is limiting.

For biomass production in temperate wetlands, there is extensive experimental evidence showing that P is most often limiting if the N:P ratio of the above-ground biomass is greater than 15, whereas N or both N and P are limiting if the N:P ratio is smaller than 15 (Verhoeven *et al.* 1996; Güsewell & Koerselman 2002; Güsewell *et al.* 2003). Occasionally, production is K-limited (when the N:K ratio is greater than 2; Olde Venterink 2000) or limited by physical factors (light or length of growing season; Spink *et al.* 1998).

A threshold N:P ratio might also discriminate between N and P limitation of litter decomposition (when the latter is not energy-limited), but the value of this threshold is so far unknown. Litter N:P ratios of 9 (Smith 2002), of 15 (Aerts 1997) or of 22 (Güsewell & Verhoeven, submitted manuscript) have been proposed. However, even the decomposition of litter with a N:P ratio of 103 may be N-limited (Güsewell, submitted manuscript). Güsewell & Verhoeven (submitted manuscript) proposed that the type of limitation might be determined not only by the N:P ratio but also by the P concentration itself; they found P limitation for litter with a P concentration below  $0.3 \text{ mg g}^{-1}$ . Conversely, Jewell (2002) observed a stimulating effect of P addition on the decomposition of grass material with a P concentration as high as  $3 \text{ mg g}^{-1}$ .

These notoriously inconsistent results might be related to the fact that nutrient availability can affect litter decomposition in multiple ways (Agren *et al.* 2001). On the one hand,

decomposers can obtain their nutrients either from the litter or from the surrounding medium (soil or water). On the other hand, mechanisms through which variation in nutrient availability can influence decomposition include effects on microbial growth, microbial carbon use efficiency, shifts in carbon quality, and the activity of enzymes involved in litter breakdown (Amador & Jones 1993; Carreiro *et al.* 2000; Agren *et al.* 2001; Thirukkumaran & Parkinson 2002). The effects of nutrients on these various components may be synergistic, independent or antagonistic. To understand and predict when N or P limits the decomposition rate of plant material, individual mechanisms and their interactions should be considered (Sinsabaugh *et al.* 1993). The present research project is concerned with one of these mechanisms, enzyme activity.

### Enzyme activity and nutrient limitation of decomposition

Assays of enzyme activity have long been used to investigate microbial activity (Frankenberger & Dick 1983; Sinsabaugh 1994) and nutrient status (Sinsabaugh *et al.* 1993; Sinsabaugh 1994). The activity of cellulolytic enzymes, e.g. exocellulase and  $\beta$ -glucosidase, often correlates closely with the initial mass loss of plant litter (Sinsabaugh & Linkins 1993). The activity of N- or P-mineralising enzymes may or may not correlate with litter mass loss because it is also influenced by nutrient availability in soil. When compared across sites, phosphatase activity may be negatively related to P availability (Gage & Gorham 1985; Kang & Freeman 1999), and the activity of N-mineralising enzymes (protease, chitinase, urease etc.) may be negatively related to N availability (Chróst 1991; Olander & Vitousek 2000). Furthermore, fertilisa-

tion with N or P can suppress the activity of N- or P-mineralising enzymes (Olander & Vitousek 2000; Colvan *et al.* 2001; Dilly & Nannipieri 2001), and fertilisation with N can increase the activity of phosphatase (Gage & Gorham 1985; Olander & Vitousek 2000; Dilly & Nannipieri 2001).

Relationships between enzyme activities and nutrient availability in soil have suggested that microbes adjust their enzyme production to nutrient availability so as to acquire C, N and P in proportions similar to those needed for growth – insufficient supply of N or P would therefore lead to an increased production of N- or P-mineralising enzymes (Sinsabaugh *et al.* 1993; Sinsabaugh & Moorhead 1994; Olander & Vitousek 2000). Such adjustments might explain the effects of nutrient availability on decomposition rates. Sinsabaugh *et al.* (1993) proposed a model for an enzyme-mediated regulation of wood decomposition by N and P availability in soil. A modified version of their model (for the regulation of leaf litter decomposition by litter N and P contents) is shown in Fig. 1. The model assumes that decomposition rates and microbial production mainly depend on the activity of C-mineralising enzymes. The synthesis of these enzymes in turn depends on microbial biomass and on how microbes partition their

Respiration

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graph TD
    LitterMassLoss[Litter mass loss] --> LabileC[Labile C]
    LabileC --> Respiration[Respiration]
    LabileC --> MicrobialProduction[Microbial production]
    MicrobialProduction --> CellulolyticEnzymes[Cellulolytic enzymes]
    CellulolyticEnzymes --> LitterMassLoss
    MicrobialProduction --> Phosphatases[Phosphatases]
    Phosphatases --> LabileP[Labile P]
    LabileP --> MicrobialProduction
    LitterPContent[Litter P content] --> LitterNP[Litter N:P ratio]
    LitterNContent[Litter N content] --> LitterNP
    LitterNP --> LabileN[Labile N]
    LabileN --> MicrobialProduction
    MicrobialProduction --> ProteaseAmidase[Protease Amidase]
    ProteaseAmidase --> LitterNContent
    MicrobialProduction --> LitterMassLoss
    MicrobialProduction --> Respiration
    
```

**Fig. 1.** Conceptual model of possible relationships between litter N and P contents, enzyme activity and litter mass loss during the initial decomposition of leaves from herbaceous plants. The rate of litter mass loss during initial decomposition depends on the activity of cellulolytic enzymes. The synthesis of these enzymes depends on microbial production and on their resource allocation to the synthesis of various enzymes. If microbes optimise their resource allocation so as to balance their C, N and P acquisition, N or P limitation should result in an increased synthesis of N- or P-mineralising enzymes. This diversion of resources from the synthesis of cellulolytic enzymes would result in a reduced rate of litter mass loss. Modified from Sinsabaugh *et al.* (1993).

**Table 1.** Hypothesised relationships between elemental ratios in plant litter or ratios of enzyme activities and the element(s) which are limiting for litter decomposition. The anticipated thresholds were derived from previous experiments, literature, and data in Fig. 2. Limitation by C means by labile C, as a source of energy. We assume no other element than C, N or P to be limiting.

Predictor variable	Anticipated Threshold (T)	Limiting element	
		Above T	Below T
Litter C:N ratio	25–30	N or P	C or P
Litter C:P ratio	500–1000	P or N	C or N
Litter N:P ratio	15–25	P or C	N or C
$\beta$ -Glucosidase/amidase	<1 (?)	C or P	N or P
$\beta$ -Glucosidase/phosphatase	<2 (?)	C or N	P or N
Amidase/phosphatase	<2 (?)	N or C	P or C

resources among different enzymes. If microbes adjust their enzyme production to nutrient supply, low nutrient concentrations in litter should stimulate the synthesis of N- and P-mineralising enzymes, and the litter N:P ratio should determine which enzymes are produced preferentially. As a result, resources would be diverted from the synthesis of C-mineralising enzymes, which would reduce the rate of litter mass loss.

According to the model in Fig. 1, N limitation of litter decomposition is expected to reduce the activity of C-mineralising and P-mineralising enzymes by reducing both microbial production and microbial resource allocation to these enzymes. In contrast, the effect of N limitation on N-mineralising enzymes could be either positive or negative as microbial production would be reduced while the relative allocation of resources to N-mineralising enzymes would be enhanced. For the same reason, P limitation could either enhance or reduce the activity of P-mineralising enzymes. This uncertainty could be avoided by considering the activities of different enzymes relative to each other. Relative enzyme activities would be independent of microbial biomass and may therefore directly reflect how microbes partition their enzyme production.

In our research project we will test this model by investigating relationships between the C, N and P concentrations of the litter, the nutrient limitation of decomposition, and the activities of enzymes involved in the mineralisation of C, N and P. We hypothesise that the type of nutrient limitation is determined by C:N:P ratios of the litter, and that it is reflected by the relative activities of C-, N-, and P-mineralising enzymes (Table 1). Based on these assumptions, our aim is to determine thresholds between N and P limitation. We expect some of the thresholds to be species-specific and others valid more generally for herbaceous wetland plant litter. Such general thresholds or combinations of thresholds could be used in carbon cycling models.

In this note we first present the results of a preliminary experiment in which we tested whether nutrient addition does influence enzyme activity during the decomposition of nutrient-rich and nutrient-poor plant material in a way that is consistent with the model shown in Fig. 1. We then describe the main experiment of the project, which will directly relate enzyme activities to litter nutrient concentrations and nutrient limitation of decomposition, using many litter types from different wetland plant species.



**Table 2.** Enzymes included in our experiments with their natural substrates, the substrates used in enzyme assays (MUF = 4-Methylumbelliferyl), and the ranges of product (4-Methylumbelliferone) concentrations obtained after 2 h of incubation. In our experiments, a product concentration of 20  $\mu\text{M}$  corresponds to an enzyme activity of 1  $\mu\text{mol h}^{-1} \text{g}^{-1}$  (initial air-dry litter mass).

Enzyme	Natural substrate	Assay substrate	Product
$\beta$ -Glucosidase	Cellobiose	MUF- $\beta$ -D-glucoside (400 $\mu\text{M}$ )	0–50 $\mu\text{M}$
Amidase	Amides	MUF-glucoside-amide (400 $\mu\text{M}$ )	0–50 $\mu\text{M}$
Phosphatase	Organic P monoesters	MUF-phosphate (200 $\mu\text{M}$ )	0–20 $\mu\text{M}$

### Preliminary experiment:

#### Effects of nutrient addition on enzyme activities

##### METHODS

Twenty plants of *Carex elata* were grown in the greenhouse in potting soil for two years. Their leaves were harvested in November 2002, pooled for all plants, and subdivided into entirely green and entirely senesced parts, discarding the rest. For simplicity, this material is called “litter” in the following. The N and P concentrations of green leaves (determined in Kjeldahl digests) were 7.6 mg N  $\text{g}^{-1}$  and 1.4 mg P  $\text{g}^{-1}$  (based on litter dry mass), and those of senesced leaves were 3.5 mg N  $\text{g}^{-1}$  and 0.47 mg P  $\text{g}^{-1}$ . The air-dried litter was incubated at 22 °C in Petri dishes on 18 g of sand covered with a 300  $\mu\text{m}$  polyethylene mesh (Verseidag Techfab, Geldern, Germany). The sand was wetted with 8 ml of water collected in a flooded meadow co-dominated by *C. elata* to inoculate the samples with appropriate decomposing microbes. Four nutrient treatments were created by adding 12 mg N (as  $\text{NH}_4\text{NO}_3$ ), 2 mg P (as  $\text{KH}_2\text{PO}_4$ ), both N and P, or no nutrients (control) to the water. The N+P treatment was only applied to senesced leaves for lack of material from green leaves.

Enzyme activity was determined after five weeks of incubation following the general recommendations of Sinsabaugh *et al.* (1991),

adapted in such a way that the activities of  $\beta$ -glucosidase, amidase and phosphatase could be assayed for each sample simultaneously with closely similar procedures (cf. Freeman & Nevison 1999). The litter was blended with 10 ml of deionised water and mixed for 30 sec in a stomacher. Aliquots of the resulting microbial suspension were transferred to six 1.5-ml centrifuge vials. Solutions of three different substrates were added, i.e. one substrate per enzyme (Table 2). Each substrate was added to two vials; the relative volumes of microbial suspension and substrate solution (ml per vial) were 0.5:1 for  $\beta$ -glucosidase but 1:0.5 for amidase and phosphatase.

After 1 h of incubation at 22 °C, the vials were centrifuged for 5 min at 10'000  $\text{min}^{-1}$  to interrupt the reaction. The reaction product (4-Methylumbelliferone) was analysed immediately on a fluorescence spectrometer (Fluostar Galaxy, BMG Lab-technologies, Offenburg, DE) with solutions of 4-Methylumbelliferone (MUF) as standards.

Because the concentration of the reaction product was directly proportional to enzyme activity per litter sample, these concentrations and their ratios were used here as relative measures of enzyme activity. The effects of plant material (green vs. senesced leaves) and nutrient treatments (control, +N, +P) on the activity of each enzyme and their ratios were analysed with two-way Anova. The N+P addition treatment was not included in these

**Table 3.** Results of two-way Anova testing the effects of litter type (green vs. senesced leaves) and nutrient treatments (control, +N, +P) on the activity of three enzymes and their ratios after five weeks of decomposition ( $G = \beta$ -Glucosidase;  $A =$  Amidase;  $P =$  Phosphatase). The N+P-addition treatment was not included in these analyses as it was only applied to senesced leaves. The number of residual df differs among variables because the enzyme assays included in calculations (cf. Methods) were partly carried out on different litter samples; for the same reason, differences in the  $G/A$  ratio could not be tested statistically. Significance levels are \*\*\*,  $P < 0.001$ ; \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ; no symbol,  $P > 0.05$ .

	df	G	A	P	G/P	A/P
Litter type	1	20.2***	13.7**	2.6	52.0***	21.2***
Treatment	2	16.8***	5.7*	39.5***	0.8	0.8
Litter x Treat	2	1.7	2.4	1.3	2.0	3.9*
Residual df		17	28	35	16	28

analyses as it had only been applied to senesced leaves. The effects of nutrient treatments were further compared pairwise for each of the two litter types with Tukey-Kramer tests.

Six replicate samples per litter type and nutrient treatment were normally assayed, but some samples were assayed with different volumes of microbial suspension or different incubation times to further develop the assay method. The activities thus obtained were excluded from data analysis, so that the actual number of replicates for which results are shown (Fig. 2) ranged from 1 to 6.

## Results and discussion

The activities of  $\beta$ -glucosidase and amidase differed significantly between the two litter types, being higher on green than on senesced leaves, while the activity of phosphatase did not differ (Table 3). The effects of nutrient treatments were similar for all three enzymes: their activity was enhanced by fertilisation with N or with N+P, the effect of N being more pronounced with the senesced leaves than with green ones; addition of P alone had no effect on any of the enzymes (Fig. 2a-c). The ratio of  $\beta$ -glucosidase to amidase activity did not differ among litter types or treatments

(Fig. 2d), whereas the ratio of  $\beta$ -glucosidase to phosphatase activity and the ratio of amidase to phosphatase activity were higher on green than on senesced leaves (Fig. 2e,f). Additionally, the  $\beta$ -glucosidase/phosphatase activity ratio was increased by fertilisation with N+P (but not with N or P alone; Fig. 2e), and the amidase/phosphatase activity ratio on green leaves was higher after fertilisation with P than after fertilisation with N (Fig. 2f).

The results of this experiment are consistent with other studies showing that the activities of ubiquitous enzymes such as  $\beta$ -glucosidase, amidase and phosphatase correlate with the microbial biomass and their respiratory activity (Frankenberger & Dick 1983; Sinsabaugh & Linkins 1993). Thus, the higher activity of  $\beta$ -glucosidase and amidase on green than on senesced leaves reflects the faster decomposition of green leaves: in separate incubations, mass loss after ten weeks (mean  $\pm$  SE,  $n = 4$ ) was  $42.6\% \pm 2.3\%$  for the green leaves and  $15.8\% \pm 0.2\%$  for the senesced ones (S. Güsewell, unpublished data).

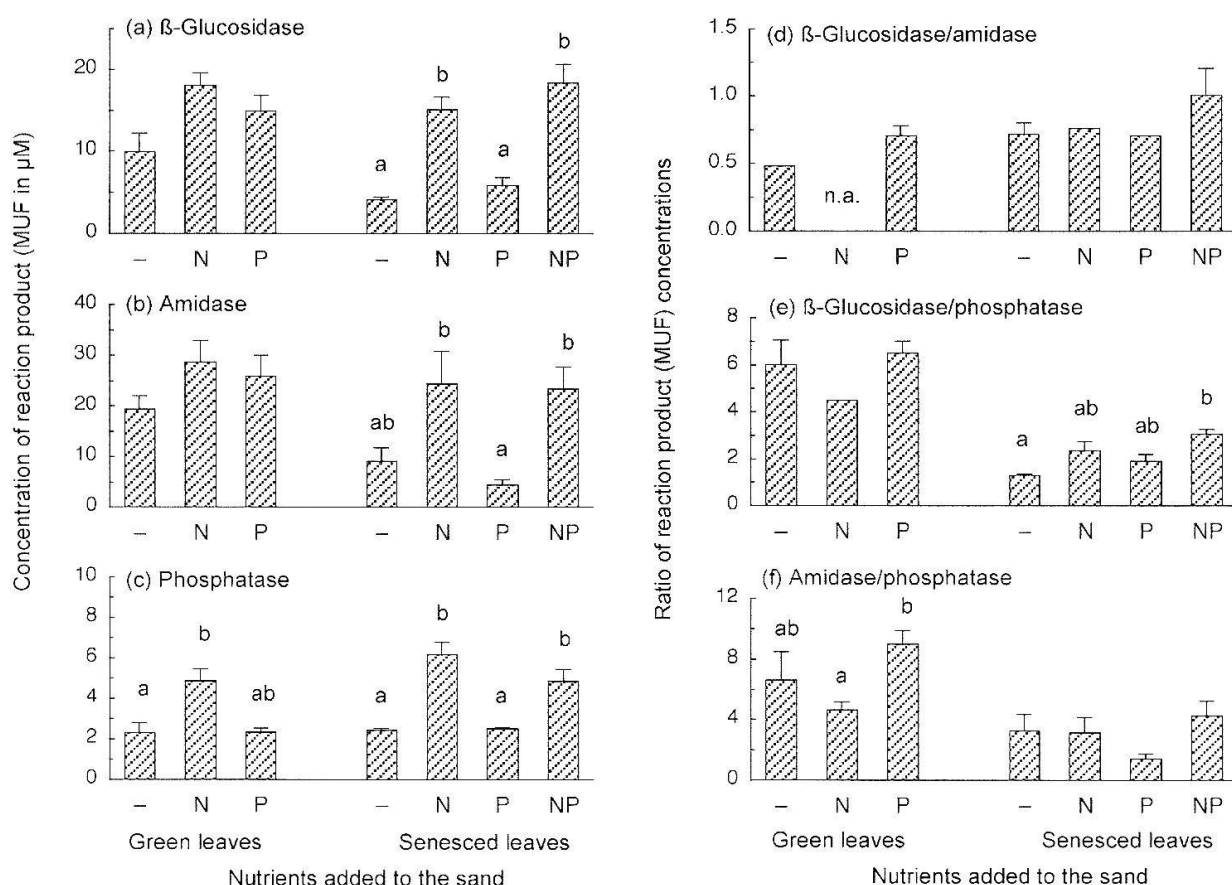
Nutrient treatments had basically the same effects on the activities of all three enzymes, which were enhanced by the addition of N. This probably reflects the fact that decomposition rate was limited by N and not by P, as suggested by the very low N:P ratios of the



plant material (5.7 for green leaves, 7.5 for senesced leaves). The absence of P limitation was further shown directly in separate incubations: mass loss after ten weeks on P-fertilised sand was  $40.8\% \pm 2.4\%$  for green leaves and  $16.18\% \pm 0.2\%$  for senesced leaves, i.e. not different from mass loss on unfertilised sand (see above). N limitation was not tested directly here, but decomposition of litter from *Carex elata* plants grown under the same conditions was N-limited in a previous experiment (Güsewell, submitted manuscript).

Our finding that the  $\beta$ -glucosidase/amidase activity ratio was not reduced by N fertilisa-

tion was unexpected, but it might be explained by the very low N:P ratio of the plant material. Indeed, if we assume that the litter N concentration after ten weeks of incubation was at most doubled by N fertilisation, as found in previous experiments (S. Güsewell, unpublished data), the N:P ratios would still be at most 15 for both litter types in the +N treatment, suggesting that C or P was still not limiting for decomposition (Güsewell & Verhoeven, submitted manuscript). Thus, according to our hypotheses (Table 1), no considerable shift in relative enzyme activities was to be expected. It is clear, however, that our hypotheses need to be tested further in



**Fig. 2.** Effects of nutrient addition on enzyme activity during the decomposition of green and senesced leaves of *Carex elata*. The concentrations of reaction products are shown as measures of enzyme activity in a–c, and ratios of these concentrations are shown as measures of relative enzyme activities in d–f. Data are means  $\pm$  SE,  $n = 1$ –6 (cf. Methods). For green and senesced leaves separately, significant differences among treatments are indicated by different letters (Tukey Kramer test,  $P < 0.05$ ); no letters means that treatment effects were not significant. Treatments are –, control; N, addition of nitrogen; P, addition of phosphorus; NP, addition of N and P.

experiments that include litter with P-limited decomposition. We expect this to be the case with the main experiment described hereafter.

### Main experiment: Effects of plant species and litter nutrients on enzyme activities

The four steps of the experiment are as follows:

- Six wetland species (forbs, grasses and sedges) are grown at differing N and P supplies, and their leaf litter is collected.
- The activity of three enzymes is determined during the initial decomposition of each litter type
- The C, N and P concentrations are determined for each litter type
- For each litter type we test whether decomposition is N- or P-limited

Nine plant species from fens or wet grasslands (*Carex elata*, *C. flava*, *Alopecurus pratensis*, *Anthoxanthum odoratum*, *Holcus lanatus*, *Molinia caerulea*, *Phalaris arundinacea*, *Centaurea angustifolia*, *Mentha aquatica*) are grown in pots with sand and fertilised with nutrient solutions that supply N and P in mass ratios of 5, 10, 20, 40, 80 and 160, with six replicates per treatment (in total 216 plants). The selection of species was based on previous experiments showing that their N and P concentrations and N:P ratios are differently high and respond differently to variation in the N:P supply ratios. The lowest N:P supply ratio during plant growth is 5 because previous experiments showed that lower N:P supply ratios result in poor plant growth and little litter production, but not in reduced litter N concentration.

Cultivation started in April 2003 and will last until full shoot senescence. Freshly senesced leaves will be collected every two weeks starting in July 2003 and air-dried. For

the enzyme assays, two replicate subsamples (150 mg) of litter from each plant will be incubated at 22 °C in Petri dishes (as in the preliminary experiment). The start of incubations will be spread over time in such a way that the duration until enzyme tests is the same for all litter types, viz. three weeks for one of the replicates and six weeks for the other replicate. Methods for enzyme assays will be as described for the preliminary experiment but 1 ml of microbial suspension will be used for all three enzymes and the duration of incubation will be extended to 2 h to obtain more pronounced differences between litter types.

Further subsamples of each litter type will be used to determine the N and P concentrations (modified Kjeldahl digestion followed by colorimetric analysis) and the C and N concentrations (on an elemental analyser). The analysis of C concentrations may be limited to some samples of each species if these concentrations prove to be independent of treatments. The nutrient limitation of decomposition will be determined by comparing the litter mass loss after 10 weeks of incubation at 22 °C with and without N ( $\text{NH}_4\text{NO}_3$ ) or P ( $\text{KH}_2\text{PO}_4$ ) fertilisation.

Regressions and analyses of covariance will be used to investigate relationships between the effects of N or P fertilisation on litter mass loss and the enzyme activity ratios as well as the litter C:N, C:P and N:P ratios, both within and across species. If, as we expect, most litter types have either N- or P-limited decomposition, discriminant analysis will be used to determine the combinations of predictor variables that discriminate best between the two types of limitation.

### Relevance of the expected results

Our research should contribute to a better understanding of when and how changed nu-

trient availability in soil affects litter decomposition, and possibly, to the ability to predict such effects. If we find relationships between relative enzyme activities and the nutrient limitation of decomposition, as hypothesised and as suggested by the results of our preliminary experiment, further research will be needed to assess whether these relationships can also be used to predict the effects of changed nutrient availability on decomposition rates under field conditions. Two issues appear critical:

First, enzyme activity in soils is strongly influenced by climatic factors and soil pH (Eivazi & Tabatabai 1988; Kang & Freeman 1999) as well as by interactions between enzymes and the soil matrix or plant roots (Tarafdar & Jungk 1987; Sinsabaugh 1994; Moorhead & Linkins 1997; Moorhead *et al.* 1998). The calculation of enzyme ratios should eliminate some of the climatic effects, yet enzymes have different activation energies and may therefore respond differently to variation in temperature (Eivazi & Tabatabai 1988). Enzyme-soil or enzyme-root interactions are even more specific (Moorhead & Linkins 1997). If the regulation mechanisms proposed in our conceptual model (Fig. 1) are strict, relationships between enzyme activity ratios and nutrient limitation might also hold under field conditions. This would require that microbes adjust their enzyme synthesis to the availability of N and P and to soil conditions, so that the resulting enzyme activities would provide balanced nutrient flows. However, it is uncertain whether such a strict regulation occurs.

Second, the present project deals with the initial stages of decomposition, during which microbial activity is highest and nutrient effects most pronounced (Coûteaux *et al.* 1995). During this phase, microbial breakdown focuses on soluble compounds and unlignified cellulose (Heal *et al.* 1997). Further research

should consider the later stages of litter decomposition, dominated by the breakdown of lignin and lignified cellulose (Berendse *et al.* 1987; Aber *et al.* 1990), which are most relevant for the long-term carbon balance of ecosystems.

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