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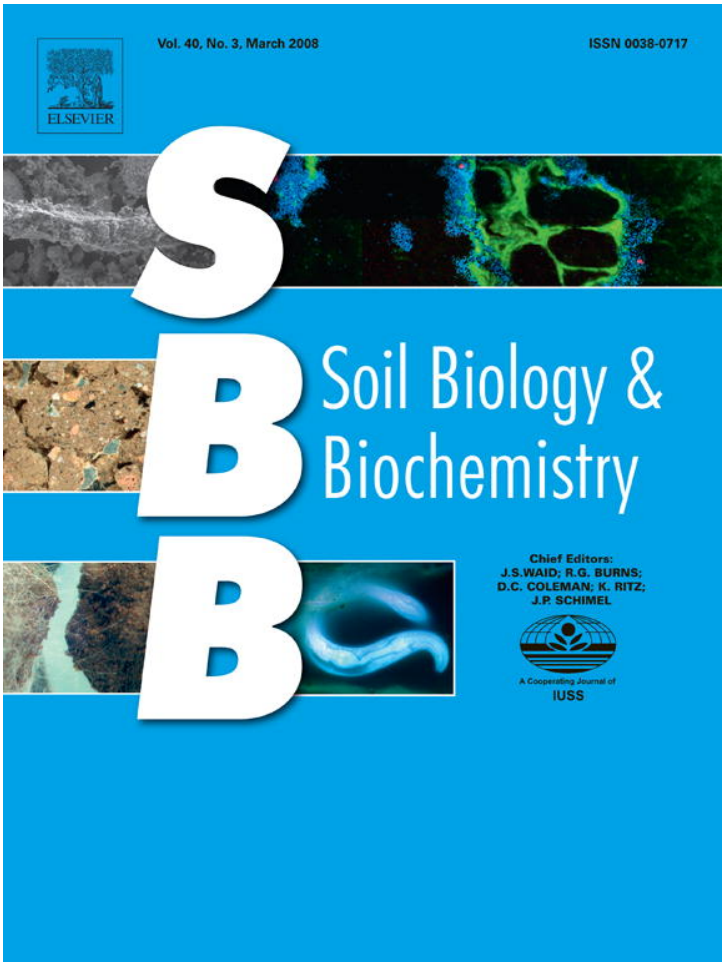


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# Direct and indirect effects of nitrogen deposition on litter decomposition

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**Abstract**

Elevated nitrogen (N) deposition can affect litter decomposition directly, by raising soil N availability and the quantity and quality of litter inputs, and indirectly by altering plant community composition. We investigated the importance of these controls on litter decomposition using litter bags placed in annual herb based microcosm ecosystems that had been subject to two rates of N deposition (which raised soil inorganic N availability and stimulated litter inputs) and two planting regimes, namely the plant species compositions of low and high N deposition environments. In each microcosm, we harvested litter bags of 10 annual plant species, over an 8-week period, to determine mass loss from decomposition. Our data showed that species differed greatly in their decomposability, but that these differences were unlikely to affect decomposition at the ecosystem level because there was no correlation between a species' decomposability and its response to N deposition (measured as population seed production under high N, relative to low N, deposition). Litter mass loss was ~2% greater in high N deposition microcosms. Using a comprehensive set of measurements of the microcosm soil environments, we found that the most statistically likely explanation for this effect was increased soil enzyme activity (cellobiosidase, $\beta$-glucosidase and $\beta$-xylosidase), which appears to have occurred in response to a combination of raised soil inorganic N availability and stimulated litter inputs. Our data indicate that direct effects of N deposition on litter input and soil N availability significantly affected decomposition but indirect effects did not. We argue that indirect effects of changes to plant species composition could be stronger in natural ecosystems, which often contain a greater diversity of plant functional types than those considered here.
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*Keywords:* Nitrogen deposition; Litter decomposition; Soil enzyme activities; PLFA; C:N ratio; Plant species composition; Decomposer community

## 1. Introduction

The process of decomposition is vital in regulating ecosystem carbon (C) storage and nutrient cycling (Wardle, 2002), and the rate at which litter decomposes is known to depend upon soil nitrogen (N) availability (Fog, 1988; Knorr et al., 2005). Because human alteration of the N cycle has resulted in an approximate doubling of terrestrial N inputs (Vitousek et al., 1997), this relationship has potentially important implications for the global carbon cycle.

Studies to date have generally shown that the decomposition rate of high quality (i.e. with low lignin content and/or narrow C:N ratio) litter is stimulated by elevated N deposition, but that the decomposition of low quality litter is retarded (Waldrop et al., 2004; Knorr et al., 2005). However, at the ecosystem scale, the relationship between N input rates and decomposition is likely to be far more complex than that observed in simple experiments in which N is added to soil in the absence of living plants (for examples, see Fog, 1988). The reason for this is that N fertilisation affects decomposer organisms both directly and indirectly. Here, we classify direct effects as greater inorganic N availability, stimulation of plant biomass production (Gough et al., 2000; Shaver et al., 2001) and

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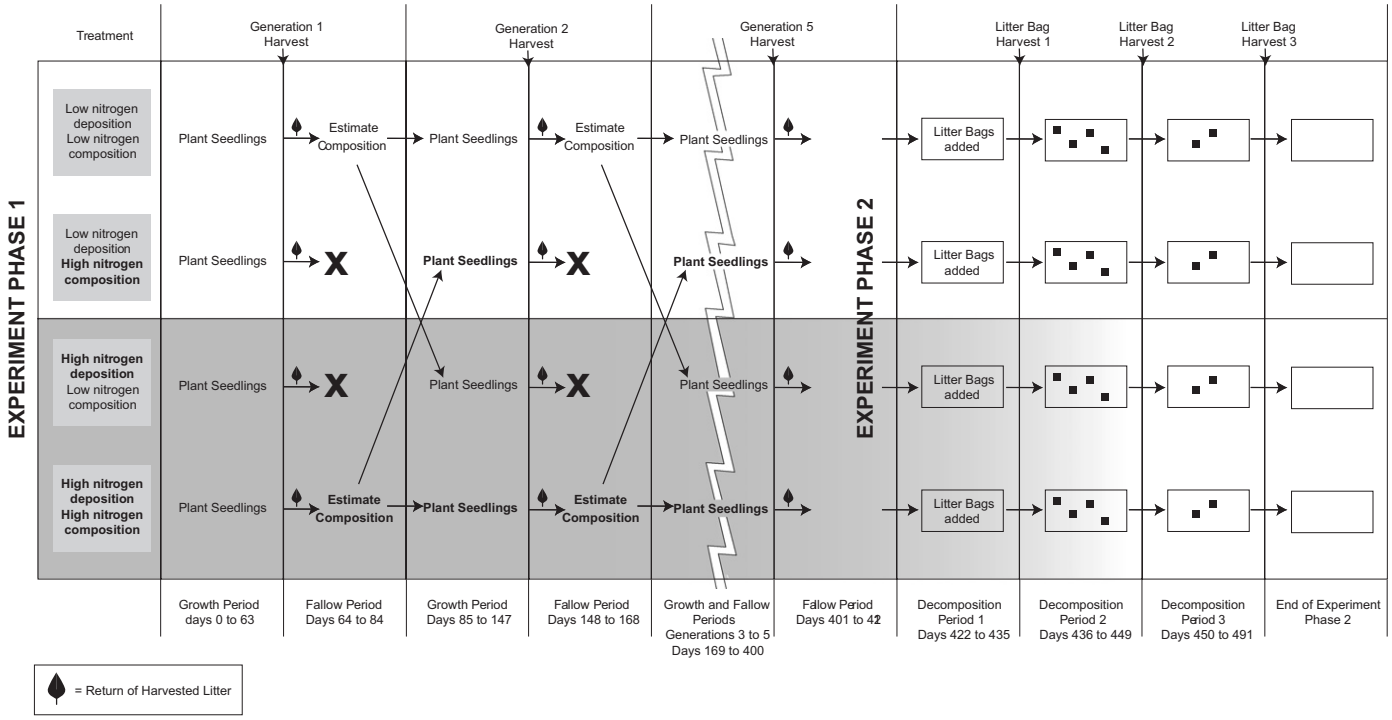


Fig. 1. Design and timeline of the experiment. In the first phase seedlings were planted, grown for 11 weeks and then harvested. The harvested biomass in two of the treatments was used to predict the composition of the next generation. The broken line indicates that the same pattern of planting, harvesting and composition estimation continued over generations 3–5. In the second phase, litter bags were added to the microcosms and harvested at 2, 4 and 8 weeks.

litter inputs, and decreased litter C:N ratio (e.g. Henry et al., 2005). An example of such a direct effect would be the stimulation of decomposer abundance and activity (potentially both microbes and larger soil organisms) by increased C and N inputs resulting in more rapid litter decomposition. We classify indirect effects as those that operate via plant species differences in response to the additional N, resulting in changes to plant species composition. This often shifts plant community composition towards rapidly growing species and these tend to produce high quality litters which decompose rapidly (Suding et al., 2005). Together, these changes mean that high N deposition systems can receive greater and higher quality litter inputs compared to unfertilised ecosystems, and that the species composition, and hence quality, of these inputs will also differ. These processes may be further complicated by the fact that an ecosystem's capacity to decompose plant litter may depend upon previous litter inputs, because they can affect soil physicochemical properties and the activity and composition of decomposer communities (Zak et al., 2003; Porazinska et al., 2003). Earlier litter inputs from a particular species for instance, may cause an increase in the population size of decomposer species (e.g. microarthropods) that are particularly suited to consume that litter species, thus accelerating its decomposition in the future relative to other species.

Here, we describe a two-phase experimental study in which we unravelled direct and indirect effects of N deposition on litter decomposition. We did this in model ecosystems (microcosms) based upon an agricultural field margin community containing eight species of annual herbs. In the first experimental phase, we separated direct and indirect effects by planting the plant species composition of low and high N environments into model ecosystems (indirect effects) that were subject to both low and high rates of N deposition (direct effects) (Manning et al., 2006) (Fig. 1). After five plant generations, soil properties were quantified, and the environments generated by the treatments, which differed in decomposer abundance and activity, pH and N availability, were used as the basis of the second experimental phase. In this phase, we placed litter bags of the eight species (plus two other species with no recent litter input history) into our model ecosystems and measured the effects of the treatments on the decomposition of litter (Fig. 1). We expected direct effects of N deposition on decomposition to operate through stimulation of the decomposer community by increased inorganic N inputs and increased litter C and N inputs. We expected indirect effects of N deposition to operate via the effects of changes in plant composition on the decomposer community, which might feedback to alter rates of decomposition. More specifically, we expected to observe correlations between: (a) plant species response to N in experimental phase one and its litter decomposition rate; and (b) the previous abundance of a plant species in a plot and its litter decomposition rate.

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## 2. Materials and methods

### 2.1. Study system

The experiment was conducted in the Ecotron Controlled Environment Facility at Silwood Park (Lawton, 1996). The Ecotron comprises 16 chambers, in which climatic and biotic conditions can be closely controlled. Within each of these was a microcosm ecosystem with a surface area of 1.09 $m^2$, containing a sandy loam soil with a depth of 240 mm. Drainage was facilitated by placing the soil atop a gravel layer 110 mm deep. Each microcosm contained a full, standardised soil community; microcosms were initially part-sterilised with methyl bromide ($CH_3Br$) and then inoculated with microbes (according to the method of Jones et al., 1998), plants, mesofauna and mycorrhizal fungi from an agricultural field margin habitat. These were added, respectively, 18, 39, 56 and 39 days after sterilisation. One species of earthworm, *Lumbricus rubellus* (Hoffmeister), was also added 56 days after sterilisation. The plant species used were a community of eight $C_3$ annual herb species, which co-exist in agricultural field margin communities (OV9d, UK National Vegetation Classification; Rodwell, 2000). They were: *Marticaria recutita* (L.), *Matricaria discoidea* (DC.), *Tripleurospermum inodorum* ((L.) Schultz-Bip), *Sonchus asper* (L.), *Solanum nigrum* (L.), *Senecio vulgaris* (L.), *Viola arvensis* (Murr.) and *Papaver dubium* (L.). Such communities are likely to vary greatly in their N inputs depending on the local use of fertiliser and proximity to heavily populated areas. The establishment of these microcosms is described by Manning et al. (2006). Initial soil characteristics, averaged across all microcosms, were (mean ± S.E.M.): pH 6.82 ± 0.04; total N (% w/w) 0.16 ± 0.005; total C 1.80 ± 0.07; dissolved inorganic N (DIN) (mg N $kg^{-1}$) 2.49 ± 0.21 and, available phosphorus (mg P $kg^{-1}$) 14.57 ± 0.42. The soil was 2% fine gravel (2–4 mm), 2% coarse sand (0.5–2.1 mm), 37% medium sand (0.05–0.5 mm), 44% silt (0.002–0.05 mm) and 15% clay (< 0.002 mm). Conditions simulated a diurnal cycle with a 16 h day peaking at 22 °C (S.D. = 0.2) and declining over an 8 h night to 12.3 °C (S.D. = 0.2). Relative humidity varied between 83% and 63%.

### 2.2. Experimental design

#### 2.2.1. Phase 1

In the first experimental phase, we applied two treatments over five plant generations (Fig. 1). These treatments, namely N deposition and plant species composition, were applied to the microcosms in a factorial design, giving four replicates per treatment combination. For the N deposition treatment, we applied two levels of ammonium nitrate ($NH_4NO_3$) as wet deposition in the daily rainfall: low N deposition, 0.2 g N $m^{-2}$ $y^{-1}$, S.E.M. = 0.04; and, high N deposition, 4.4 g N $m^{-2}$ $y^{-1}$, S.E.M. = 0.1). This allowed us to determine the direct effects of N deposition. These rates correspond to 2.0 and 44.0 kg N $ha^{-1}$ $y^{-1}$, if we assume that one generation of our experiment is a surrogate to 1 year. Although we advise caution when comparing these values with those of natural ecosystems, the comparison is not entirely unrealistic as many processes which occur over several years in natural ecosystems (e.g. growth, senescence and decomposition) are condensed into a single generation in our system. These rates are comparable to wet deposition rates in Western Europe and North America (Holland et al., 2005).

In the second treatment we manipulated plant community composition, by controlling the relative planted abundance of the eight species, to determine indirect effects. This treatment had two levels, namely low N composition and high N composition, which simulated either plant community change across the five generations, under either the low N deposition regime or the high N deposition regime. The relative abundance of each plant species at the start of each new plant generation was determined by the relative biomass of plant species at the end of each previous generation. At the end of each 11-week generation (coinciding with the onset of senescence), aboveground biomass was destructively harvested, dried at 60 °C and weighed (Fig. 1). We then used these biomass data to estimate the seed output produced in each microcosm using calibrated models which predict seed production as a function of plant biomass (see Manning et al., 2006 for further details). Of the 216 seedlings planted into each microcosm in each generation we always planted a pair of individuals of each of the eight species; this represented immigration from a regional species pool. The remaining 200 seedlings were divided amongst the eight species according to their proportional contribution to community seed mass in the previous generation. Communities were initially even, with 27 individuals of each species. After the first harvest microcosms experiencing low N deposition and which had always been planted with a low N community composition were used to estimate the low N community composition. Those experiencing high N deposition, and which had always been planted with a high N community composition, were used to estimate the high N community composition (see Fig. 1 and Manning et al., 2006 for further details).

When harvesting plant biomass all root material was left to decompose in the soil with the exception of ten 25 mm diameter and 100 mm deep soil cores from which roots were extracted by washing, dried at 60 °C and weighed. Extrapolating these estimates generated root biomass production estimates for the top 100 mm of soil. In the 3-week fallow periods that separated generations 50% of the harvested aboveground material was returned as litter to its microcosm of origin (Fig. 1). At the end of the fifth generation, immediately prior to biomass harvesting, a wide range of soil properties associated with litter decomposition were measured in each microcosm (Fig. 1) using the following methods.

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Soil samples were taken to a depth of 100 mm and the activity of several enzymes involved in C mineralisation, namely cellobiosidase (exo-1,4-$\beta$-glucanase, E.C. 3.2.1.91), *N*-acetyl-glucosaminidase (E.C. 3.2.1.30), $\beta$-glucosidase (E.C. 3.2.1.21) and xylosidase (E.C. 3.2.1.37), were measured according to the method of Marx et al. (2001). This was achieved using a computerised microplate fluorimeter with fluorogenic methylumbelliferone (MUB) substrates (respectively): 4-MUB-$\beta$-D-cellobioside, 4-MUB-*N*-acetyl-$\beta$-glucosaminide, 4-MUB-$\beta$-D-glucoside and 4-MUB-7-$\beta$-D-xyloside. Furthermore, xylanase activity and invertase activity (E.C. 3.2.1.26) were colorimetrically determined according to Schinner and von Mersi (1990).

Microbial biomass C was estimated using the chloroform fumigation extraction technique (Vance et al., 1987) and the abundance of phospholipid fatty acids (PLFA), which was determined by extracting PLFAs from soil, and then fractionating and quantifying them according to the method described by Bardgett et al. (1996). Separated fatty acid methyl-esters were identified by chromatographic retention time and mass spectral comparison using a standard qualitative bacterial acid methyl-ester mix and fatty acid methyl-ester mix (Fa. Supelco) that ranged from C11 to C20. Individual PLFA signatures were grouped into two categories, fungal PLFA (18:2$\omega$6) and bacterial PLFA (the sum of i15.0, a15:0, i16.0, 17:0, i17:0, cy17:0, 18:1$\omega$7 and cy19:0), and the ratio of fungal:bacterial PLFAs was calculated (Bardgett and McAlister, 1999). Nomenclature of PLFAs followed Frostegård et al. (1993). The density of Collembola was estimated by taking cores from each microcosm and extracting with a Tullgren funnel, and the rate of N mineralisation was measured using in-situ cores that were sampled at 22 and 71 days into the fifth (final) generation of experimental phase one (see Manning et al., 2006 for details of both methods). Measures of DIN concentrations (extracted with 2 M KCl and measured immediately using colorimetric methods), pH (in deionised water) and bulk density were taken at the end of each generation from subsamples of the soils used to measure enzyme activity, PLFAs and microbial biomass C. We also measured the total soil C and N using a total combustion analyser and the amount of soil C in three different fractions using physical separation after chemical dispersal. The fractions measured were coarse particulate organic matter (CPOM) ($>0.5$ mm), fine particulate organic matter (FPOM) (0.5 mm to 53 µm) and mineral associated C ($<53$ µm).

After the harvest of the fifth generation, 50% of aboveground biomass was returned as litter in the fallow period, as before, but N deposition halted and seedlings were not replanted (Fig. 1). Over the course of experimental phase one, the plant species community composition treatment diverged significantly. By the fifth generation the low N composition treatment was dominated by one species, *M. recutita*, while the high N composition was dominated by three *S. nigrum*, *M. recutita* and *S. asper*. The high N deposition treatment had strongly stimulated the quantity of litter inputs via enhanced biomass production, and had increased litter quality by reducing litter C:N ratio (see Manning et al., 2006 for a detailed description of the effects of both treatments). Therefore, there were strong differences in the litter input histories experienced by each treatment. These are summarised in Table 1.

### 2.2.2. Phase 2

The second experimental phase began after the fifth and final fallow period (Fig. 1), by which point the returned litter had been decomposing for 14 days. We then added litter bags ($7\times7$ cm bags with pore size of 2 mm, each containing 0.6 g of litter) to the surface of the microcosms, in direct contact with the soil. These litter bags belonged to two treatments applied to the bags in a factorial design within each microcosm. The first treatment was litter type, which had 18 levels; 16 of these comprised the eight aforementioned plant species of the first experimental phase, each present at two C:N levels. These two levels of litter quality were generated by using litter that had been grown in low N deposition conditions ($0.2\,g\,N\,m^{-2}\,generation^{-1}$) or grown in high N conditions ($4.4\,g\,N\,m^{-2}\,generation^{-1}$). The remaining two levels of the litter type treatment were bags containing litter of one of two species, either *Chenopodium album* (L.) or *Lamium purpureum* (L.). These species co-exist with the experiment phase one species in the OV9d community but have no history of litter input into the microcosms. These were present at one C:N level. There were six bags of each litter type treatment in each of the 16 microcosms. All litter was finely crushed and mixed material that was derived from senescing shoot material and oven dried at 60 °C. Litter for most species originated from the first generation of experimental phase one. This choice was based on the known differences in the C:N ratio of the litter, previously found within the N deposition treatment. The C:N ratio of the litter was calculated from measures of litter C and N content measured by total combustion (Dumas technique) on subsamples of homogenised litter. For most species, there was sufficient litter from the harvested material of the first generation to make up all the required bags (i.e. $>70$ g). Where there was insufficient material from the first generation of phase one material was taken from other generations or another experiment (Feedbacks Experiment) based upon the same study system and rates of N application. For the two plant species with no previous litter input history, *C. album* and *L. purpureum*, all litter was taken from another experiment, again based on the same study system (Trial experiment). The origin and C:N ratio of the litters used in the experiment are summarised in Table 2.

The second treatment was time, which comprised destructive harvests of two litter bags from each microcosm at 2, 4 and 8 weeks. Therefore, there were two replicates of

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Table 1
Total litter inputs ± S.E.M. into the microcosms over the last three generations of experiment phase one (i.e. the inputs which are most likely to account for differences in the soil environment)

| | Low N deposition, low N composition | Low N deposition, high N composition | High N deposition, low N composition | High N deposition, high N composition |
|---|---|---|---|---|
| Total litter input ($g\,m^{-2}$) | 692.2 ± 12.7 | 711.9 ± 20.6 | 1018.8 ± 33.1 | 1021.6 ± 41.9 |
| Shoot litter input[a] | 588.6 | 589.7 | 818.5 | 779.8 |
| Root litter input[b] | 103.6 ± 12.7 | 122.2 ± 20.6 | 200.3 ± 33.1 | 241.9 ± 41.9 |
| Total litter C input ($g\,m^{-2}$)[c] | 391.8 | 368.5 | 494.8 | 477.2 |
| Total litter N input ($g\,m^{-2}$)[c] | 6.4 | 6.3 | 12.2 | 11.5 |
| C:N ratio[c] | 61.8 | 63.1 | 41.2 | 42.5 |
| Contribution to shoot litter input (%) | | | | |
| *Matricaria discoidea* | 0.6 | 0.4 | 0.4 | 0.3 |
| *Matricaria recutita* | 54.4 | 32.2 | 40.3 | 20.8 |
| *Papaver dubium* | 0.1 | 0.1 | 0.2 | 0.1 |
| *Senecio vulgaris* | 3.1 | 2.8 | 3.1 | 3.1 |
| *Solanum nigrum* | 2.8 | 11.5 | 8.8 | 17.3 |
| *Sonchus asper* | 37.1 | 50.7 | 46.0 | 56.9 |
| *Tripleurospermum inodorum* | 1.8 | 2.0 | 0.9 | 1.2 |
| *Viola arvensis* | 0.2 | 0.3 | 0.3 | 0.3 |

Note that much of the additional litter N input in the high N deposition treatment may have resulted from the nitrogen additions that these microcosms had received.
All means are derived from four replicates ($n = 4$).
[a]Shoot input = 50% of mean aboveground biomass harvested in this treatment (the amount that was returned as litter).
[b]Root input = 100% of root dry biomass at the time of harvest.
[c]Total C and N input estimates were derived from separate measurements of root and shoot C and N contents that were obtained using digestion techniques.

Table 2
Origin and C:N ratio of the litter used in the litter type treatment

| Litter species | Origin (%) | | C:N | |
|---|---|---|---|---|
| | Low N | High N | Low N | High N |
| *Matricaria recutita* | Generation 1 (100%) | Generation 1 (100%) | 44.21 | 30.82 |
| *Papaver dubium* | Generation 1 (100%) | Generation 1 (100%) | 32.26 | 27.11 |
| *Senecio vulgaris* | Generation 1 (100%) | Generation 1 (100%) | 31.69 | 22.98 |
| *Solanum nigrum* | Generation 1 (100%) | Generation 1 (100%) | 40.99 | 31.09 |
| *Viola arvensis* | Generation 1 (100%) | Generation 1 (100%) | 37.10 | 25.12 |
| *Sonchus asper* | Generation 1 (92%)<br>Generation 3 (8%) | Generation 1 (100%) | 52.80 | 42.57 |
| *Matricaria discoidea* | Generation 1 (80%)<br>Feedbacks experiment (20%) | Generation 1 (87%)<br>Feedbacks experiment (13%) | 25.86 | 20.02 |
| *Tripleurospermum inodorum* | Generation 1 (25%)<br>Feedbacks experiment (75%) | Generation 1 (25%)<br>Feedbacks experiment (75%) | 34.9 | 23.81 |
| *Chenopodium album* | Trial experiment (100%) | Trial experiment (100%) | 63.79 | NA |
| *Lamium purpureum* | Trial experiment (100%) | Trial experiment (100%) | 27.06 | NA |

Litter material of *Chenopodium album* and *Lamium purpureum* were grown at a single N supply rate and so were of a single C:N ratio.

each litter type × time combination at the microcosm level (but 32 in total) and 108 litter bags per microcosm (1728 in total).

Litter bags were placed on the soil surface in a stratified random pattern. Soil moisture varied across the surface of the microcosms and so soil moisture conditions under each bag were recorded when bags were harvested, using a theta probe (Delta-T devices, Cambridge, UK). Harvested bags were oven dried at 60 °C and weighed to estimate mass loss (decomposition).

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Table 3
Effect of N deposition and plant species composition treatments on the soil environment at the end of the first experimental phase

| Response variable | Low N deposition ($n = 8$) | High N deposition ($n = 8$) | N deposition | | Plant species composition | | N deposition × plant species composition | |
|---|---|---|---|---|---|---|---|---|
| | | | $F_{1,12}$ | $P$ | $F_{1,12}$ | $P$ | $F_{1,12}$ | $P$ |
| Total soil carbon (% w/w) | 1.71 (0.12) | 1.89 (0.03) | 1.44 | 0.25 | 0.0 | 0.84 | 0.0 | 1.0 |
| Total soil nitrogen (% w/w) | 0.143 (0.008) | 0.168 (0.004) | 6.4 | 0.03 | 0.0 | 0.97 | 0.3 | 0.6 |
| FPOM C ($\mu g\,C\,g^{-1}$) | 2.60 (0.09) | 3.47 (0.12) | 28.8 | $\leqslant 0.001$ | 0.4 | 0.54 | 0.5 | 0.5 |
| pH | 6.94 (0.04) | 6.70 (0.04) | 14.5 | $\leqslant 0.001$ | 0.2 | 0.64 | 1.0 | 0.3 |
| N mineralization ($\mu g\,N\,g^{-1}\,d^{-1}$) | 0.09 (0.02) | 0.14 (0.05) | 1.0 | 0.34 | 0.7 | 0.43 | 0.7 | 0.4 |
| Dissolved inorganic N availability ($\mu g\,N\,g^{-1}$) | 1.91 (0.26) | 3.08 (0.18) | 17.2 | $\leqslant 0.001$ | 4.0 | 0.07 | 1.9 | 0.2 |
| *N*-Acetyl-glucosaminidase activity ($nmol\,g^{-1}\,h^{-1}$) | 138 (7) | 213 (13) | 30.6 | $\leqslant 0.001$ | 3.8 | 0.08 | 0.5 | 0.5 |
| $\beta$-Glucosidase activity ($nmol\,g^{-1}\,h^{-1}$) | 196 (8) | 246 (15) | 8.3 | 0.01 | 0.5 | 0.49 | 0.0 | 0.9 |
| Cellobiosidase activity ($nmol\,g^{-1}\,h^{-1}$) | 30.3 (1.7) | 41.3 (4.5) | 5.9 | 0.03 | 1.0 | 0.33 | 3.2 | 0.1 |
| Invertase activity ($\mu g\,GLC\,g^{-1}\,3\,h^{-1}$) | 2721 (184) | 3382 (93) | 9.1 | 0.01 | 0.3 | 0.60 | 0.2 | 0.7 |
| Xylanase activity ($\mu g\,GLC\,g^{-1}\,24\,h^{-1}$) | 813 (28) | 1259 (50) | 87.4 | $\leqslant 0.001$ | 2.8 | 0.12 | 2.7 | 0.1 |
| $\beta$-Xylosidase activity ($nmol\,g^{-1}\,h^{-1}$) | 45.2 (1.3) | 56.0 (4.3) | 4.9 | 0.05 | 1.4 | 0.26 | 0.1 | 0.8 |
| Collembola density 0–50 mm ($n\,m^{-2}$) | 3959 (715) | 8206 (1491) | 5.9 | 0.03 | 3.5 | 0.08 | 0.0 | 1.0 |
| Microbial biomass C ($\mu g\,C\,g^{-1}$) | 126 (6) | 142 (5) | 4.2 | 0.06 | 0.5 | 0.49 | 0.7 | 0.4 |
| Total PLFA ($nmol\,g^{-1}$ soil) | 42.2 (2.2) | 60.3 (2.5) | 27.2 | $\leqslant 0.001$ | 0.1 | 0.74 | 0.8 | 0.4 |
| Total fungal PLFA (18:2ω6) ($nmol\,g^{-1}$ soil) | 1.57 (0.09) | 2.18 (0.15) | 11.0 | 0.006 | 0.0 | 0.88 | 0.5 | 0.5 |
| Total bacterial PLFA ($nmol\,g^{-1}$ soil) | 14.8 (0.8) | 21.7 (0.9) | 26.6 | $\leqslant 0.001$ | 0.2 | 0.69 | 0.8 | 0.4 |
| Fungal:bacterial PLFA ratio | 0.11 (0.0) | 0.10 (0.01) | 0.5 | 0.50 | 0.2 | 0.69 | 0.0 | 0.9 |

All means are pooled across the composition treatment and are therefore derived from eight replicates ($n = 8$). Values in parentheses = S.E.M.

### 2.3. Statistical analysis

All analysis was performed in S-Plus for Windows v.6.1 (Venables and Ripley, 2002). Effects of the N deposition and plant species composition treatments on the soil environment at the end of phase one were assessed using two-way analysis-of-variance (ANOVA). Effects of the experimental treatments and soil moisture on litter decomposition were assessed using a linear mixed-effects (lme) model (Pinheiro and Bates, 2000). The litter type treatment was split into a categorical factor, litter species, and a continuous variable, C:N ratio, which was treated as a covariate. The random effects structure of this model was microcosm within block. We also tested the hypothesis that species selected by high N deposition are more decomposable. This was achieved by regressing the mean mass loss of each species after 8 weeks (averaged across all other treatments) upon the response of each plant species to N deposition in experimental phase one. The number of seedlings planted in each level of the composition treatment was a function of a species' proportional contribution to community seed mass in the high N deposition, high N composition and low N deposition, low N composition treatments and treatment combinations (see above and Fig. 1). Therefore, species response to N deposition was calculated by taking the mean of differences in the number of planted seedlings between the two composition treatments over the last three generations of experimental phase one.

Analysis of the experimental data revealed significant effects of N deposition on decomposition (see Section 3). In order to get a better understanding of the mechanisms underlying the direct effect of N deposition on litter decomposition we took a model selection approach (Pinheiro and Bates, 2000; Johnson and Omland, 2004) in which we compared the likelihood of several competing statistical models. The null model was a reduced version of that originally used to investigate treatment effects and contained only the significant terms: soil moisture, time, litter species, N deposition, soil moisture × time and time × litter species (see Section 3). In subsequent versions of this model, we substituted the N deposition term with each of the continuous soil property variables that were significantly affected by the N deposition treatment in experimental phase one (Table 3). The likelihood of the competing models were compared using Akaikies Information Criterion (AIC); which provides information on how likely a model is, given the data and its parameterisation. In comparing two alternate models that with the lower AIC is the more likely. In addition to this analysis, we also attempted to assess the nature of the direct N deposition effect by performing the same analysis on data that had been scaled relative to soil FPOM C content, but only for measures of soil decomposer activity and abundance. Soil FPOM C (the soil C fraction utilised by most soil decomposer organisms) increased significantly under high N deposition (see Section 3 and Table 3). It is therefore

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conceivable that the changes in soil decomposer abundance and activity, and subsequently litter decomposition rate, which occurred in response to N deposition, could be due to increased N availability, increased C availability or a combination of the two. By dividing the measures of soil decomposer activity and abundance by soil FPOM C content, we corrected for the effect of higher C availability. Although caution is advised, due to strong covariance in all measures affected by the N deposition treatment, significance of these variables in the reduced model after correction for FPOM C content does indicate an additional effect of higher N availability on the decomposer community and subsequently decomposition.

## 3. Results

### 3.1. Treatment effects on the soil environment

At the end of experimental phase one, there was a significant negative effect of N deposition on pH, and significant positive effects on the following variables: total soil N, FPOM C, DIN, soil enzyme activities (*N*-acetyl-glucosaminidase, *β*-glucosidase, cellobiosidase, invertase, xylanase and xylosidase), collembolan density, fungal PLFA, bacterial PLFA and total PLFA (Table 3). Variables which did not show significant responses to N deposition were: total soil C, rate of N mineralisation, microbial biomass C and the fungal:bacterial PLFA ratio (Table 3). There were no significant effects of plant species composition on any of the measured variables and no significant interactions between plant species composition and N deposition (Table 3).

### 3.2. Controls of decomposition

Litter species differences in decomposition rate were particularly strong ($F_{9,1641} = 287.9$, $P \leqslant 0.001$); mass loss after 2 weeks varied from 57.7% for the fastest decomposing species *V. arvensis*, to 27.4% for the slowest, *C. album* (Fig. 2). However, the regression between species response to N deposition (i.e. the difference in the number of planted seedlings between the composition treatments) and decomposition rate was not significant ($F_{1,6} = 0.0$, $P > 0.05$, $r^2 = 0.0$). Therefore, the hypothesis that N deposition selects for more rapidly decomposing species was not supported.

Decomposition was strongly affected by soil moisture ($F_{1,1641} = 102.9$, $P \leqslant 0.001$) and by time ($F_{2,1641} = 479.9$, $P \leqslant 0.001$). That is, mass loss was approximately 4.5% greater per 10% increase in soil moisture content, and increased by 11.4% between weeks 2 and 4, and by a further 4.7% between weeks 4 and 8 (Fig. 2). There was

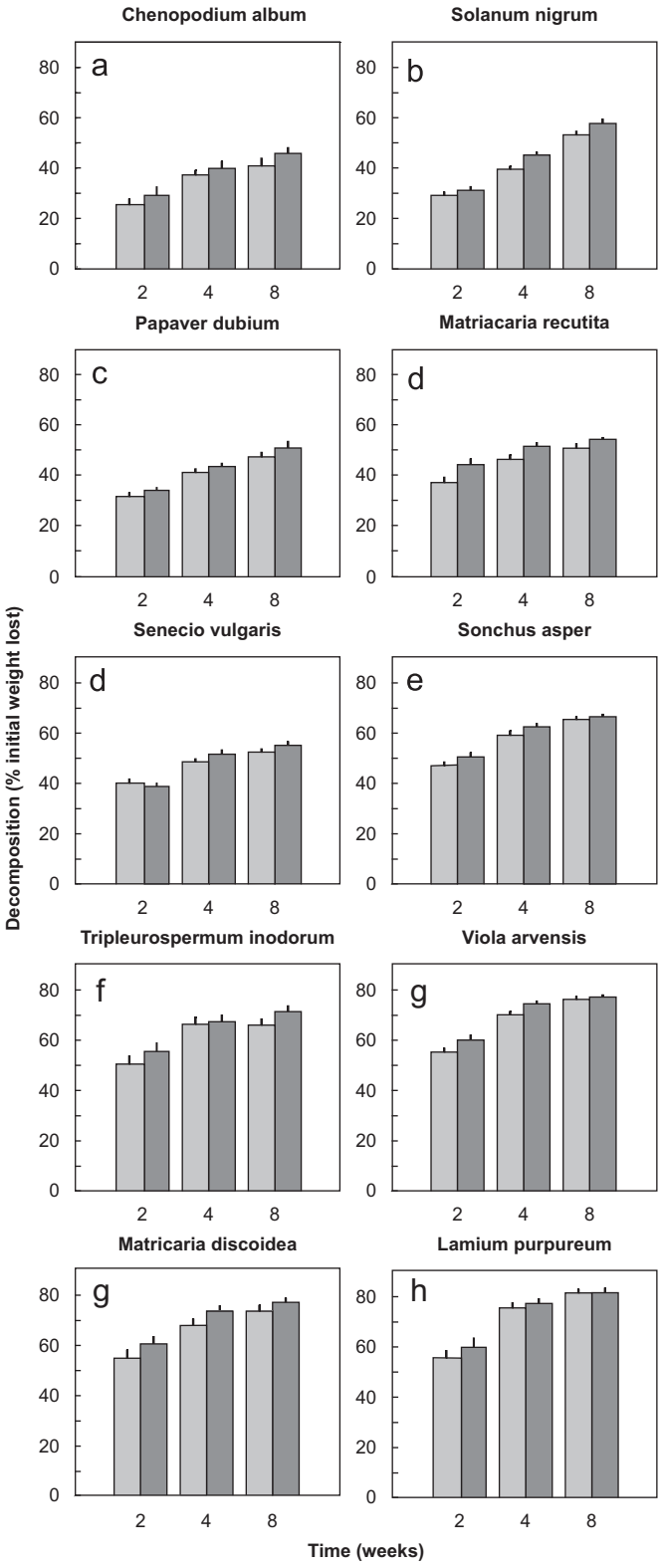


Fig. 2. Decomposition of 10 annual herb species over 8 weeks in microcosms subject to two histories of N deposition. Light shaded bars = low N deposition history. Dark shaded bars = high N deposition history. Each bar is derived from 64 replicates ($n = 64$, with the exception of *L. purpureum* and *C. album* ($n = 32$). Species share the same letters when their temporal pattern of decomposition did not differ significantly at the $<0.05$ level. This was assessed using 6 d.f. likelihood ratio tests in which the full model (as presented in Table 4) was compared to a model in which two of the species terms were combined. This was done for all possible two species combinations. Error bars represent the S.E.M.

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also a significant interaction between time and litter species ($F_{9,1641} = 3.2$, $P \leqslant 0.001$), reflecting the fact that the more slowly decomposing species continuously lost mass between 2 and 8 weeks (e.g. *C. album*, *S. nigrum* and *P. dubium*), while the more decomposable species decomposed little between 4 and 8 weeks (e.g. *M. discoidea*, *V. arvensis* and *T. inodorum*) (Fig. 2).

Table 4
Analysis of variance for treatment and covariate effects on litter decomposition

| Variable | $F_{d.f.}$ | $P$ |
|---|---|---|
| Intercept | $987.1_{1,1641}$ | <0.0001 |
| Soil moisture | $103.0_{1,1641}$ | <0.0001 |
| Plant species composition | $0.1_{1,9}$ | 0.81 |
| N deposition | $17.9_{1,9}$ | 0.002 |
| Time | $479.9_{2,1641}$ | <0.0001 |
| Litter species | $287.9_{9,1641}$ | <0.0001 |
| C:N | $1.8_{1,1641}$ | 0.18 |
| Soil moisture × plant species composition | $0.1_{1,1641}$ | 0.83 |
| Soil moisture × N deposition | $0.3_{1,1641}$ | 0.59 |
| Soil moisture × time | $3.7_{2,1641}$ | 0.03 |
| Soil moisture × litter species | $1.6_{9,1641}$ | 0.10 |
| Soil moisture × C:N | $1.8_{1,1641}$ | 0.18 |
| Plant species composition × N deposition | $0.5_{1,9}$ | 0.51 |
| Plant species composition × time | $0.2_{2,1641}$ | 0.80 |
| Plant species composition × litter species | $1.5_{9,1641}$ | 0.14 |
| Plant species composition × C:N | $0.2_{1,1641}$ | 0.68 |
| N deposition × time | $0.5_{2,1641}$ | 0.63 |
| N deposition × litter species | $0.5_{9,1641}$ | 0.85 |
| N deposition × C:N | $0.1_{1,1641}$ | 0.82 |
| Time × litter species | $3.2_{18,1641}$ | <0.0001 |
| Time × C:N | $0.3_{2,1641}$ | 0.74 |

Because analysis was performed using a linear mixed-effects model $P$ values should be considered approximate.

The interaction between soil moisture and time was also significant ($F_{2,1641} = 3.7$, $P = 0.026$) (Table 4) and reflects a shift in the relationship between soil moisture and mass loss over time. In the fitted model, an increase of soil moisture content from 0.1 to 0.4 g g$^{-1}$ increases mass loss by 11.1% 2 weeks into decomposition, but by only 7.6% after 8 weeks.

Litter decomposition was typically ~2% greater in high N deposition microcosms (Fig. 2), an effect that was highly significant ($F_{19} = 17.9$, $P \leqslant 0.001$) (Table 4), but small in comparison to litter species, moisture and time effects. The nonsignificance of the N deposition × time interaction indicates that greater mass loss in the high N deposition treatment occurred during the first 2 weeks and was typically conserved until the end of the experiment. Plant species composition, litter quality (C:N ratio) and all interactions involving the litter input history factors had no effect on mass loss (Table 4). The interaction between litter species and the species composition treatment was not significant (Table 4), suggesting that species did not decompose more rapidly in microcosms where they had previously been an abundant litter source.

### 3.3. Mechanisms underlying the N deposition effect

Comparison of the models competing to explain the N deposition effect found that the most likely explanation was increased activity of soil enzymes involved in C mineralisation (Table 5). Of these, the most likely was cellobiosidase activity (AIC = 12,707, $F = 21.31$, $P < 0.001$), which was increased by N deposition by 36.1% (Tables 3 and 5). Other likely variables were β-xylosidase

Table 5
Explaining the N deposition effect on litter decomposition

| Variable substituted | Original data | | | FPOM C corrected data | | |
|---|---|---|---|---|---|---|
| | AIC | $P$ | $F_{1,11}$ | AIC | $P$ | $F_{1,11}$ |
| N deposition (null model) | 12,711 | ** | 15.87 | NA | NA | NA |
| pH | 12,717 | * | 4.4 | NA | NA | NA |
| Dissolved inorganic N availability | 12,720 | n.s. | 1.2 | NA | NA | NA |
| *N*-Acetyl-glucosaminidase activity | 12,710 | ** | 16.2 | 12,720 | ** | 7.2 |
| β-Glucosidase activity | 12,713 | ** | 11.9 | 12,721 | n.s. | 0.4 |
| Cellobiosidase activity | 12,707 | *** | 21.3 | 12,720 | * | 4.6 |
| Invertase activity | 12,718 | * | 4.1 | 12,720 | n.s. | 0.0 |
| Xylanase activity | 12,715 | * | 7.4 | 12,721 | n.s. | 2.2 |
| β-Xylosidase activity | 12,710 | ** | 16.4 | 12,721 | n.s. | 0.6 |
| Collembola density ($\log y + 1$) | 12,719 | n.s. | 2.4 | 12,721 | n.s. | 0.1 |
| Total PLFA | 12,716 | * | 5.5 | 12,720 | n.s. | 0.8 |
| Total fungal PLFA (18:2ω6) | 12,719 | n.s. | 2.2 | 12,719 | 0.6 | 0.6 |
| Total bacterial PLFA | 12,721 | n.s. | 0.4 | 12,717 | * | 5.2 |

Variables that were significantly affected by N deposition (N dep.) in the last generation of experiment phase one were substituted for the N deposition term in the reduced model. This permitted us to assess how likely an explanation of N deposition effects they were. The $P$ and $F$ values presented relate to the significance of the substituted variable within the reduced model. The FPOM corrected data columns show the likelihood of the model and the significance of the substituted term when it is scaled relative to soil FPOM C content, an analysis that was performed for measures of decomposer abundance and activity measures only. n.s.: $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

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activity (AIC = 12,710, $F = 16.38$, $P < 0.01$), which was increased by 23.8% under high N deposition, and *N*-acetyl-glucosaminase activity (AIC = 12,710, $F = 16.22$, $P < 0.01$) (Tables 3 and 5), which was increased by 54.2% (Table 5). Less likely were terms representing the effects of DIN, lower pH, microbial biomass, soil fauna and the abundance of fungi or bacteria, as represented by PLFA signatures (Tables 3 and 5). Variables which were likely descriptors of the N deposition effect were not always those which showed the strongest responses to N deposition, e.g. DIN, bacterial PLFA and fungal PLFA (Tables 3 and 5). The analysis in which soil measures of soil decomposer abundance and activity were scaled to soil FPOM C content suggests that the observed direct effect was mainly due to the effects of increased litter inputs, as opposed to higher N availability. That is, with the exception of *N*-acetyl-glucosaminase activity ($F = 7.2$, $P < 0.01$), cellobiosidase activity ($F = 4.6$, $P < 0.05$) and bacterial PFLA abundance ($F = 5.2$, $P < 0.05$) no variables displayed a significant relationship with litter decomposition after scaling to FPOM C content (Table 5). Significance of these latter three factors after scaling for FPOM C content, however, suggests that their increases were disproportional to increases in soil C, thus indicating that their effect on decomposition was also due to other direct effects of N deposition (e.g. high soil N availability). Note that it is not possible that the N deposition effect was due to enrichment of litter N content from wet deposition as N deposition stopped before the litter bags were placed.

## 4. Discussion

We expected direct effects of N deposition on decomposition to operate through stimulation of the decomposer community by increased litter and N inputs and indirect effects to operate via changes in plant composition on the decomposer community, which feedback to alter rates of decomposition. In our system however, only the direct effect of N deposition on N availability and litter inputs were detected.

One of the strongest controls of decomposition in our study (alongside time and soil moisture) was litter species identity, a finding that is consistent with many other studies (e.g. Wardle, 1997; Cornelissen et al., 1999; Smith & Bradford, 2003). Despite these strong differences, changes in plant species composition caused by N deposition are unlikely to affect decomposition at the ecosystem level in our system (assuming no strong litter mixing effects (Wardle, 1997)), because there was no correlation between species differences in decomposition and species response to N deposition. This could be due to the lack of functional diversity in our system, because all the species in the study were annual herbs. Indeed, in systems where N deposition causes dominance shifts between major functional groups (e.g. from dwarf shrubs to grasses), compositional change can have dramatic effects on the decomposability of litter inputs (Berendse et al., 1989). Although species effects were strong, litter C:N ratio did not affect decomposition in our microcosms, despite it being a good predictor of early stage decomposition in many systems (Swift et al., 1979). This suggests that other components of litter chemistry controlled decomposition in our system.

Decomposition was greater, albeit by a small amount (i.e. ~2%), in microcosms that had received higher N and plant litter inputs as a consequence of a high N deposition regime in phase one of our experiment. This effect was small compared to those of litter species and soil moisture, but was consistent in that it affected litter species similarly (Fig. 2) and implies that at least some of the C storage gains associated with greater productivity under elevated N deposition (Reich et al., 2006) might be lost via accelerated decomposition. It is likely that this effect was the combined result of multiple changes to decomposer abundance and activity. Although the fungal:bacterial ratio was not affected by N deposition, the abundance of fungi and bacteria, Collembola and the activity of soil enzymes all increased in response to this treatment, suggesting increased decomposer abundance and activity. These changes were presumably a response to the higher C and N inputs that high N microcosms received. However, they differ from those generally seen in forest, grassland and tundra systems subject to elevated N deposition, where decreases in fungal biomass and a reduction in the fungal:bacterial ratio is often observed (e.g. Bardgett and McAlister, 1999; Donnison et al., 2000; Frey et al., 2004; Schmidt et al., 2004; Smith et al., 2003; Bradley et al., 2006; de Vries et al., 2006). This discrepancy might be because the fungi that are susceptible to N addition in later successional, or old-field ecosystems (which typically have high fungal:bacterial biomass ratios (Bardgett et al., 2005)) were not present in our more early successional system.

Of the changes to the soil environment that occurred in response to elevated N deposition, the most statistically likely explanation for the N deposition effect on decomposition was increased activity of enzymes involved in C mineralisation. This hypothesis is supported by several studies in which soil enzyme activity corresponds with litter mass loss (e.g. Carreiro et al., 2001; Luxhøi et al., 2002), and by the lack of any N effect after the first 2 weeks. Although soil enzymes can be stabilised for weeks to years, activity increases in response to resource inputs are usually temporary with the enzyme decaying to its original level after substrate depletion (Stemmer et al., 1998). A second, non-exclusive explanation for the brevity of the N deposition effect might be that the factors responsible only acted upon rapidly decomposing materials (e.g. cellulose). It is generally believed that increased N availability accelerates the decomposition of rapidly decomposing substrates but retards that of slowly decomposing materials (e.g. lignin) (Waldrop et al., 2004; Knorr et al., 2005). In forest ecosystems, N additions can increase the activity of soil enzymes involved in degrading the simpler plant carbon compounds, e.g. cellulase (Carreiro et al., 2001). However, it is also known that increased litter inputs

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stimulate soil community activity (Zak et al., 2003). Our analysis, in which soil activities were scaled to soil FPOM C content, suggests that the increase in soil enzyme activities was mostly a response to increased soil C content (via higher litter inputs) but that higher soil N availability could also have played a role. The relative weakness of this effect may be due to the fact that N deposition halted before the litter bags were placed and that soil N availability could have declined in the high N deposition treatment over the second experimental phase. It is not possible that the N deposition effect was due to higher N content of the litter as N deposition stopped before the litter bags were placed.

The history of plant species composition had little effect on decomposition and there was no evidence that litter species decomposed more rapidly in soils where they had higher previous inputs. This was perhaps unsurprising considering that the plant species composition treatment had had little effect on soil properties (Table 2). This may be because the species were all annual herbs with similar chemical constituents and low concentrations of the secondary compounds that require specialist decomposers (Hättenschwiler and Vitousek, 2000). In contrast, experiments conducted in systems containing litter species with more complex chemistries (e.g. grass, shrub and tree species), suggest that decomposition rates can be affected by the history of litter inputs (Hättenschwiler et al., 2005; Milcu et al., 2006). However, another two studies conducted in microcosm (Ayres et al., 2006), and tundra systems (Hobbie and Gough, 2004) report opposing results, despite the use of chemically complex and functionally diverse litter types.

Our experiment provided an initial insight into the strengths of several mechanisms through which N deposition can alter litter decomposition. By decoupling direct effects of N deposition on plant growth and soil N availability from the indirect effects of plant community change, we have shown that the former played a more important role in determining changes to litter decomposition rate. Using statistical techniques, we were able to conclude that the underlying mechanism was most likely to be the stimulation of soil enzyme activity by increased C and N inputs. In our model system, indirect effects upon plant and decomposer communities were weak, but these may be strong in systems where plant species chemistries are more functionally diverse, and where changes to the soil environment in response to N and litter inputs are large and persistent. In such systems, a more holistic view of what controls decomposition at the ecosystem scale may be required. Therefore, a useful extension of this work would be to conduct field experiments involving the manipulation of N deposition, litter N concentration, litter quantity and litter composition across ranges that are relevant to observed rates of deposition and known ecosystem responses to N deposition. In addition, we must also increase our basic knowledge of how the quantity, form and stoichiometry of soil C and N inputs influences soil decomposer abundance and activity and how this subsequently affects decomposition, nutrient cycling and soil carbon fractionation and storage.

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