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LETTER

Decoupling the direct and indirect effects of nitrogen deposition on ecosystem function

Pete Manning,[1]* John E. Newington,[1] Helen R. Robson,[1] Mark Saunders,[1] Till Eggers,[2] Mark A. Bradford,[3] Richard D. Bardgett,[4] Michael Bonkowski,[5] Richard J. Ellis,[1] Alan C. Gange,[6] Susan J. Grayston,[7] Ellen Kandeler,[8] Sven Marhan,[8] Eileen Reid,[9] Dagmar Tscherko,[8] H. Charles J. Godfray[1] and Mark Rees[10]

Abstract

Elevated nitrogen (N) inputs into terrestrial ecosystems are causing major changes to the composition and functioning of ecosystems. Understanding these changes is challenging because there are complex interactions between 'direct' effects of N on plant physiology and soil biogeochemistry, and 'indirect' effects caused by changes in plant species composition. By planting high N and low N plant community compositions into high and low N deposition model terrestrial ecosystems we experimentally decoupled direct and indirect effects and quantified their contribution to changes in carbon, N and water cycling. Our results show that direct effects on plant growth dominate ecosystem response to N deposition, although long-term carbon storage is reduced under high N plant-species composition. These findings suggest that direct effects of N deposition on ecosystem function could be relatively strong in comparison with the indirect effects of plant community change.

Keywords

Arbuscular mycorrhizal fungi, biodiversity, evapotranspiration, frequency dependence, mineral-associated carbon, mineralization, net ecosystem productivity, plant community composition, soil decomposer community, soil enzyme activity.

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INTRODUCTION

Over the past century, nitrogen (N) inputs into terrestrial ecosystems have approximately doubled. Much of this is due to fertilizer application, although elevated deposition of N onto non-agricultural ecosystems is also widespread (Vitousek *et al.* 1997). As a result there have been major changes to ecosystem structure and function, including reductions in plant species diversity (Gough *et al.* 2000; Stevens *et al.* 2004), increases in primary productivity (Gough *et al.* 2000; Shaver *et al.* 2001), and alteration of soil carbon (C) storage and N cycling (Wedin & Tilman 1996; Neff *et al.* 2002; Mack *et al.* 2004). Understanding how these changes arise is challenging because of the complex interaction between the direct effects of N on soil chemistry and the physiology of the biota, and indirect effects of N mediated by altered species composition (Wardle 2002).

[1]*Natural Environment Research Council Centre for Population Biology, Department of Biological Sciences, Imperial College London, Silwood Park Campus, Ascot, Berkshire SL5 7PY, UK*
[2]*Ecology Division, Department for Biology and Chemistry, University of Osnabrück, Barbarastrasse 11, Osnabrück D-49069, Germany*
[3]*Institute of Ecology, University of Georgia, Athens, GA 30602, USA*
[4]*Soil and Ecosystem Ecology Laboratory, Institute of Environmental and Natural Sciences, Lancaster University, Lancaster LA1 4YQ, UK*
[5]*Technische Universität Darmstadt, Fachbereich, 10, Biologie, Darmstadt D-64287, Germany*
[6]*School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey TW20 0EX, UK*
[7]*Department of Forest Sciences, University of British Columbia, 2424 Main Mall, Vancouver, BC, Canada V6T 1Z4*
[8]*Institute of Soil Science, University of Hohenheim, 70599 Stuttgart, Germany*
[9]*Macaulay Land Use Research Institute, Aberdeen AB15 8QH, UK*
[10]*Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK*
**Correspondence: E-mail: p.manning@imperial.ac.uk*

A clear example of the way in which direct and indirect effects interact can be seen in plant community changes in response to N deposition and the effect that these have upon ecosystem function. Most plants grow larger in response to elevated N deposition, and species differences in this response results in altered community composition (Bobbink *et al.* 1998). Because plant species differ in their functional attributes (e.g. in their growth potential and tissue chemistry) compositional change can influence ecosystem function (Hooper *et al.* 2005), and modify the impact of N deposition (e.g. the high N species composition may show a greater growth response to additional N) (Bardgett *et al.* 1999; Reich *et al.* 2001; Craine *et al.* 2003; Dijkstra *et al.* 2004).

We designed an experiment to quantify the relative contributions of direct and indirect effects of elevated N deposition on a suite of ecosystem properties. The study was conducted, across five generations, in a model annual plant-based ecosystem, and consisted of two treatments, each with two levels, in a factorial design. The first treatment comprised low or high N deposition and allowed us to determine direct effects of N deposition on ecosystem functions. In the second treatment we manipulated plant community composition by planting high and low N plant species compositions into both low N deposition and high N deposition environments. This allowed us to determine indirect effects and their interaction with direct effects. By performing these manipulations we showed that ecosystem response to N deposition was dominated by direct effects upon plant growth and soil biochemistry.

METHODS

Initial soil conditions

The experiment was conducted in the Ecotron Facility at Silwood Park. The Ecotron is a controlled environment facility with replicate microcosms housed in separate chambers, in which biotic and environmental conditions can be closely controlled (Lawton 1996). In each microcosm plants were grown in a container with a surface area of 1.09 m^2 containing 10-mm diameter gravel to a depth of 110 mm, covered by 240 mm of a sandy loam soil [composed of 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)]. This soil was partially sterilized, to destroy meso- and macrofauna (> 100 μm body width diameter) and the soil seed bank, using CH_3Br fumigation, 18 days prior to placing soil in the microcosms. Before seedlings were planted, soil was flushed with repeated irrigations, over 3 weeks, to leach nutrients released by the sterilization. Initial soil characteristics were (mean ± SE): pH 6.59 ± 0.03, moisture (% w/w) 16.4 ± 0.6, total N (% w/w) 0.12 ± 0.02, total C 1.44 ± 0.02, dissolved inorganic N (DIN) (mg N kg^{-1}) 40.66 ± 3.26, available phosphorus (P) (mg P kg^{-1}) 12.19 ± 0.48. Initially high DIN values dropped to 3.60 ± 0.56 by the end of the first generation.

Community establishment

The plant community was based upon the *Matricaria perforata-Stellaria media* OV9 community of the UK National Vegetation Classification (Rodwell 2000). Eight monocarpic species were selected for their synchronous ontogeny and large range of maximum potential biomass: *Matricaria recutita*, *Matricaria discoidea*, *Papaver dubium*, *Senecio vulgaris*, *Solanum nigrum*, *Sonchus asper*, *Tripleurospermum inodorum* and *Viola arvensis* (for authorities see Anon 2006). Two-week-old seedlings were planted, at an initially even frequency (Fig. 1) at a fixed density of 198 m^{-2}, in a randomized block pattern, 2 weeks after germination. Dead individuals were replaced during the first 2 weeks of each generation.

Microbes and soil fauna were extracted from the soil of an OV9 community at Silwood Park (Berkshire, UK). Microbial addition followed the technique of Jones *et al.* (1998). Mycorrhizal fungi were added by placing root fragments from the same community under each plant individual in the first generation. Soil mesofauna (100 μm to 2 mm body width diameter) were extracted using a Tulgren funnel system, and were added to microcosms 12 days after planting the first generation. After 38 days, 40 g of fresh weight of earthworms (*Lumbricus rubellus*) was also added.

Environmental conditions simulated a diurnal cycle with a 16-h day peaking at 22 °C (SD = 0.2) with temperature declining over an 8-h night to 12.3 °C (SD = 0.2). Relative humidity varied between 83% and 63%. Photosynthetic photon flux at the soil surface, when vegetation was absent, was 210 μmol m^{-2} s^{-1} (SD = 8).

Experiment design

The study was conducted across five generations and consisted of two treatments, each with two levels, in a factorial design. The first began 2 weeks after seedlings were planted, comprised low or high N deposition and allowed us to determine direct effects of N deposition on ecosystem properties. N was applied as NH_4NO_3 at rates of 0.2 g N m^{-2} per generation (SE = 0.04) in the low N treatment, and 4.4 g N m^{-2} per generation (SE = 0.1) in the high N treatment, in the daily simulated rainfall of 4.4 mm. These rates correspond to 2.0 and 44.0 kg N ha^{-1} $year^{-1}$, 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

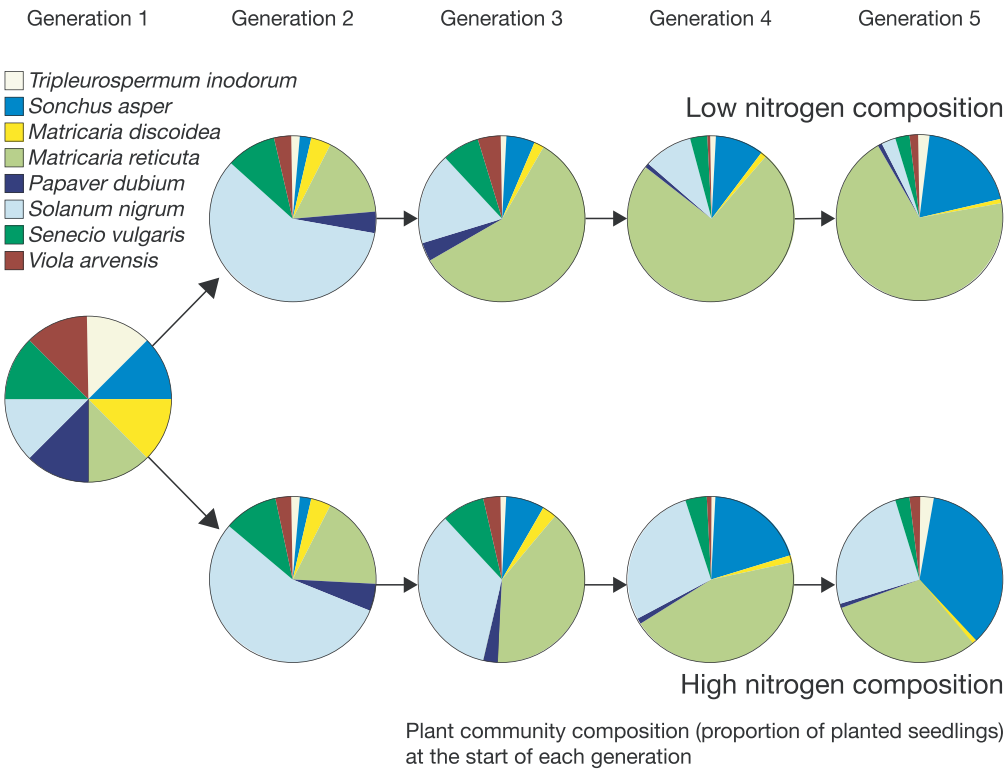

Figure 1 Plant community composition changes over the course of the experiment. Both compositions were planted into both levels of the nitrogen deposition treatment in all generations.

generation in our system. These rates are comparable with wet deposition rates in western Europe and North America (Holland *et al.* 2005).

In the second treatment we manipulated plant community composition to determine indirect effects. The species composition of each new plant generation was determined by the relative biomass of plant species at the end of the previous generation (see details below); the low N composition treatment was determined from replicates that had always experienced both low N deposition and a low N community and the high N composition treatment from replicates that had always experienced high N deposition and a high N community. Therefore, the levels of the plant composition treatment represented high and low N community trajectories. Each treatment combination was replicated four times in separate microcosms ($n = 16$). Treatments were applied in a randomized block design.

Estimation of plant community change

At the end of each 11-week generation aboveground biomass was destructively harvested, dried at 60 °C and weighed; in the subsequent 3-week fallow periods 50% of this material was returned as litter to its microcosm of origin. In addition to the aboveground biomass litter return, CH_3Br sterilized soil was added to a depth of *c.* 20 mm, during each fallow period, to prevent nutrient exhaustion and recruitment from seed. All root material was left in the soil to decompose.

During this period biomass data was used to estimate the composition of the next plant generation. This was achieved by using data from a preliminary pot experiment, in which all eight species were grown at three densities and at the two rates of N deposition, to generate statistical models of seed mass based on plant aboveground biomass (see Table S1 and Fig. S1). These models were then used to estimate the seed output of each species in each microcosm. Of the 216 seedlings planted in each microcosm per generation, 16 seedlings were reserved for pairs of individuals from each species, to represent immigration from a local species pool. The remaining 200 seedlings were divided amongst the eight species according to their proportional contribution to total community seed mass in the previous generation. As a result, species could go extinct within a generation but their populations could recover later in the experiment.

Sampling and measurement of ecosystem properties

Treatment effects on a suite of ecosystem properties associated with N, C and water cycling were measured in each generation. Soil samples were taken to a depth of

100 mm, immediately prior to biomass harvesting. Core holes were refilled with CH_3Br sterilized soil after sampling and sites were not re-sampled. Microbial biomass C was estimated using the chloroform fumigation–extraction technique (Vance *et al.* 1987). Total soil N and C were measured using a total combustion analyser. For the last generation soil organic C was physically separated, after chemical dispersal into three fractions: (i) coarse particulate organic matter (POM) (> 0.5 mm); (ii) fine POM (0.5 mm to 53 μm); and (iii) mineral-associated (< 53 μm) C (POM; > 0.5 mm). During decomposition, fresh organic matter is transformed sequentially into these fractions, which have increasing turnover times and thus potential to account for long-term C sequestration.

Activities of two soil enzymes involved in C mineralization were measured at the end of each generation. β-Xylosidase (EC 3.2.1.37) was measured using the Marx *et al.* (2001) method in a computerized microplate fluorimeter with the fluorogenic MUB-substrate (4-methylumbelliferone-7-β-D-xyloside). For determination of invertase (EC 3.2.1.26) activity, 0.5–1.0 g of soils were incubated with 5.0 mL of 50 mM sucrose solution and 5.0 mL of 2 M acetate buffer, pH 5.5 for 3 h at 50 °C. Sugars released during incubation reduced potassium hexacyanoferrate(III) in an alkaline solution and potassium hexacyanoferrate(II) was measured colorimetrically using the Prussian blue reaction (Schinner *et al.* 1996).

Root biomass and arbuscular mycorrhizal (AM) samples were taken immediately after aboveground biomass harvesting. Ten, 25-mm diameter and 100-mm deep soil cores were taken from each microcosm and roots were extracted by washing, dried at 60 °C, and weighed. For AM sampling, roots of three individuals of each species were sampled, and a section of each root system was used in estimating AM abundance [per cent root length colonized (%RLC)] with the autofluorescence method (Gange *et al.* 1999). A community level estimate of AM abundance was obtained by calculating a weighted average of species %RLC using species' aboveground biomass as weights. Collembola density was measured by taking two cores of 50-mm diameter from each microcosm and extracting from these using a Tulgren funnel for 48 h.

C-flux measurements were taken 6 weeks into each generation with a community cover box and infrared gas analyser. Day C-flux rates were determined from the difference in CO_2 concentration between air inputs and outputs in an open system. Night C-flux rates were determined from the buildup of CO_2 when the system was closed. Net ecosystem productivity (NEP) estimates (estimating total ecosystem C balance) were calculated by taking weighted averages of day and night estimates.

Nitrogen mineralization was measured using gaseously open (but hydrologically sealed) paired *in situ* cores, which were maintained at a moisture content that was consistent with the surrounding soil. Sealing prevented contamination from N deposition inputs. Cores were sampled at 22 and 71 days into each generation. Leachate volume, dissolved organic N and dissolved inorganic N were measured throughout the experiment. Soil moisture was continuously monitored with four theta probes at depths of 75 mm in each microcosm. Evapotranspiration was calculated as: I-T-C when I is rainfall input, T is through-flow and C is the change in soil water content. Hydrological data were averaged across each generation before analysis.

Statistical analysis

Compositional differences between treatments from which composition estimates were drawn were computed separately for each generation so that the timing of emergence of differences could be assessed (see Table S2 for details). Analysis of treatment effects on ecosystem properties was conducted using analysis of variance (ANOVA) or repeated measures ANOVA, depending upon whether variables had been measured regularly or in the final generation only. As it was possible that significant effects may have emerged by chance given the large number of variables measured data were also analysed with multivariate ANOVA. This was not possible for the data measured repeatedly, given the number of variables and our degrees of freedom. Block effects were not computed as preliminary analyses showed that between-block error was insignificant.

Data from the first two generations were not included in the analysis because the systems were still undergoing dramatic changes associated with their assembly. Data were $\log_e(y + 1)$ transformed to correct for heteroscedasticity for the root biomass, collembolan density and total N leaching variables. All statistical analysis was conducted using S-Plus 6 for Windows.

Hypotheses for lack of composition effects

We formulated three hypotheses, of increasing complexity, to explain the lack of compositional effects upon ecosystem function (see *Results*), and tested these with respect to plant (aboveground) biomass. Details of the assumptions underpinning each of these hypotheses can be found in Appendix S1.

The first hypothesis is that there is no differences in species per capita biomass (the species are functionally equivalent). This was tested by comparing species biomass in monoculture at both N deposition levels, using data from an independent experiment where four individuals of all eight plant species (species treatment) used in the main experiment were grown in 3-L pots at both rates of N deposition (N treatment) in glasshouse conditions until

senescence. There were 32 replicates of each treatment combination. Soil and gravel type, planting density, microbial and mycorrhizal inoculation, N and water supply rates were as in the main experiment, although the growth period was reduced to 63 days, owing to rapid plant development, and soil fauna were not introduced. Data were analysed with a general linear model with species and N terms.

Hypothesis 2 is that although species differ in per capita biomass there is no correlation between species biomass and abundance in either composition treatment. We tested this by calculating the covariance between the mean per capita biomass of each species at each N deposition level and the mean of composition differences over the last three generations, using data from both the glasshouse and main experiments. The covariance (calculated as a Pearson correlation coefficient with species weighted by their mean abundance across all replicates over the last three generations) was calculated at both levels of N deposition in both experiments. In statistical analysis these data were pooled using a Fisher's combined probability test.

Finally, we hypothesized that the per capita biomass of a species depends on its abundance in a community in a way that equalizes species biomass across composition treatments for any given level of N deposition. To test this we compared per capita and total population aboveground biomass of the dominant species across a range of frequencies by analysing data (from the last three generations of the experiment) with a linear mixed-effects model including terms for N deposition (a discrete factor), conspecific frequency (a continuous covariate), and their interaction. We also estimated community biomass in the absence of frequency dependence by multiplying the number of individuals by the mean of their species' per capita biomass in the two composition treatments, at each level of N deposition.

RESULTS

At the end of the first generation, plant biomass in all treatments was dominated by *Solanum nigrum*. Thereafter, the high and low N composition communities diverged, with two abundant species (*Solanum nigrum* and *Matricaria recutita*) differing significantly in their estimated seed production at the end of the second generation ($P < 0.001$, Fig. 1, see Table S2 for details). By the fifth generation the low N composition was dominated by one species (*Matricaria recutita*), while the high N composition was dominated by three (*Solanum nigrum*, *Sonchus asper* and *Matricaria recutita*; Fig. 1).

High N deposition had a strong, positive effect on plant biomass while the effects of high N species composition were much smaller (N deposition $F_{1,12} = 124.2$, $P < 0.001$, Table 1; composition $F_{1,12} = 0.0$, $P > 0.05$ Fig. 2). In the last three generations, 82.5% of variance in aboveground biomass was attributed to the N deposition treatment. When biomass was partitioned into aboveground and root components we found the only significant effect of species composition to be greater root biomass in the high N composition in the last generation (composition × time, $F_{2,24} = 4.8$ $P < 0.05$). However, these effects accounted for < 0.1% of the variance in total biomass (Table 1).

Because 50% of aboveground biomass and all root material were returned to the system as litter, the soil of high N deposition communities received greater inputs of organic C and N. Accordingly, a greater quantity of C and N accumulated in the topsoil of the high N deposition microcosms, compared with the low N deposition microcosms (total C, $F_{1,12} = 16.2$, $P < 0.01$, total N, $F_{1,12} = 20.0$, $P < 0.001$; Table 1; Fig. S2), but there were no effects of high N composition (total C $F_{1,12} = 0.5$, $P > 0.05$ Total N, $F_{1,12} = 0.2$, $P > 0.05$, Table 1). Increased soil C under high N deposition was also reflected in significantly greater NEP ($F_{1,12} = 10.3$, $P < 0.01$; Table 1). A number of measures reflecting decomposer activity: microbial biomass ($F_{1,12} = 26.8$, $P < 0.001$; Table 1), Collembola density ($F_{1,12} = 12.2$, $P < 0.01$; Table 1), invertase ($F_{1,12} = 37.2$, $P < 0.001$; Table 1) and β-xylosidase activity ($F_{1,12} = 8.4$, $P < 0.05$; Table 1) were significantly greater in high N deposition microcosms; again, there was no effect of high N composition on these measures ($F_{1,12} = 0$–4.7, $P > 0.05$; Table 1). N mineralization rates did not differ across treatment combinations (N deposition, $F_{1,12} = 0.0$, $P > 0.05$, composition, $F_{1,12} = 0.7$, $P > 0.05$; Table 1), and although rates of N leaching were sometimes greater in high N deposition treatments (N deposition × time, $F_{2,24} = 8.4$, $P < 0.01$, Table 1) this effect was inconsistent and leaching rates were low (total leaching averaged across all microcosms over the last three generations was 7.5 mg N m^{-2} day^{-1} SE ± 0.6).

Carbon storage in the fine POM fraction was greater under high N deposition and was unaffected by high N composition (N deposition $F_{1,12} = 28.9$, $P < 0.001$, composition, $F_{1,12} = 0.1$, $P > 0.05$, Table 1, Fig. 3a). In contrast, C storage in the mineral-associated fraction was lower in both the high N deposition and high N composition treatments (N deposition, $F_{1,12} = 9.2$, $P < 0.01$, composition, $F_{1,12} = 22.3$, $P < 0.001$ explaining 20% and 49% of the variance respectively; Table 1, Fig. 3b). AM abundance in plant roots was also significantly greater in low N species composition treatments ($F_{1,12} = 21.7$, $P < 0.001$, explaining 49% of the variance) and the low N deposition treatment ($F_{1,12} = 9.8$, $P < 0.01$, explaining 22% of the variance) (Table 1, Fig. 3c). This may be attributable to the higher abundance of *Matricaria recutita* in the low N deposition community; the mean %RLCs (± SE) of the dominant species over the whole duration of the experiment were: *Matricaria recutita* 12.0 (± 0.9), *Solanum nigrum* 11.2 (± 1.1), *Sonchus asper* 5.9 (± 0.7).

Table 1 The significance of direct nitrogen (N) deposition effects and indirect N composition effects on ecosystem properties in the last three generations*

	N dep.			Comp.			N dep. × comp.	Time‡	N dep. × time‡	Comp. × time‡
Variable	Direction of effect relative to low N dep.†	Variance explained (% total)	*P*-value	Direction of effect relative to low N comp.	Variance explained (% total)	*P*-value	*P*-value	*P*-value	*P*-value	*P*-value
Aboveground biomass (g m^{-2})	+	82.5	< 0.001	No effect	−	0.200	0.112	0.002	0.132	0.096
Log_e [root biomass (g m^{-2}) + 1]	+	12.1	0.004	No effect	−	0.137	0.923	< 0.001	0.089	0.018
Total plant biomass (g m^{-2})	+	64.5	< 0.001	No effect	−	0.976	0.532	< 0.001	0.167	0.717
Total soil C (0–100 mm) (g C m^{-2})	+	13.3	0.002	No effect	−	0.509	0.940	< 0.001	0.010	0.467
Total soil N (0–100 mm) (g N m^{-2})	+	14.8	< 0.001	No effect	−	0.670	0.809	< 0.001	< 0.001	0.787
Net ecosystem productivity (g C m^{-2} day^{-1})	+	21.0	0.007	No effect	−	0.238	0.068	0.289	0.755	0.507
Microbial biomass C (μg C g^{-1})	+	30.7	< 0.001	No effect	−	0.052	0.640	0.108	0.006	0.664
Log_e [Collembola density 0–50 mm (*n* m^{-2}) + 1]	+	24.2	0.005	No effect	−	0.128	0.664	0.171	0.249	0.979
Soil invertase activity (μg glucose equivalents g^{-1} dry weight 3 h^{-1})	+	37.2	< 0.001	No effect	−	0.542	0.715	< 0.001	0.137	0.715
Soil β-xylosidase activity (nmol g^{-1} dry weight h^{-1})	+	26.0	0.014	No effect	−	0.976	0.847	0.651	0.650	0.101
N mineralization (μg N g^{-1} day^{-1})	No effect	−	0.989	No effect	−	0.436	0.816	0.775	0.428	0.095
Log_e [total N leaching (g N m^{-2} day^{-1}) + 1]	No effect	−	0.339	No effect	−	0.473	0.572	< 0.001	0.002	0.519
Fine POM C (mg C g^{-1})	+	70.3	< 0.001	No effect	−	0.775	0.750	NA	NA	NA
Mineral-associated C (mg C g^{-1})	−	20.4	0.010	−	49.5	< 0.001	0.233	NA	NA	NA
Mycorrhizal root colonization (%)	−	22.0	0.009	−	49.0	< 0.001	0.369	NA	NA	NA
MANOVA	+/−	NA	< 0.001	−	NA	0.001	0.575	NA	NA	NA
Evapotranspiration (L m^{-2} day^{-1})	+	47.3	< 0.001	No effect	−	0.717	0.563	0.290	0.473	0.570
Soil moisture (g g^{-1})	−	59.9	< 0.001	No effect	−	0.057	0.978	< 0.001	< 0.001	0.593
Water through-flow (L m^{-2} day^{-1})	−	52.5	< 0.001	No effect	−	0.210	0.456	< 0.001	0.281	0.567

N dep., N deposition; Comp. composition; POM, particulate organic matter; NA, not applicable.

*Statistical details are in Table S3.

†+, positive effect; −, negative effect.

‡Variables with NA for time terms were measured in the last generation only.

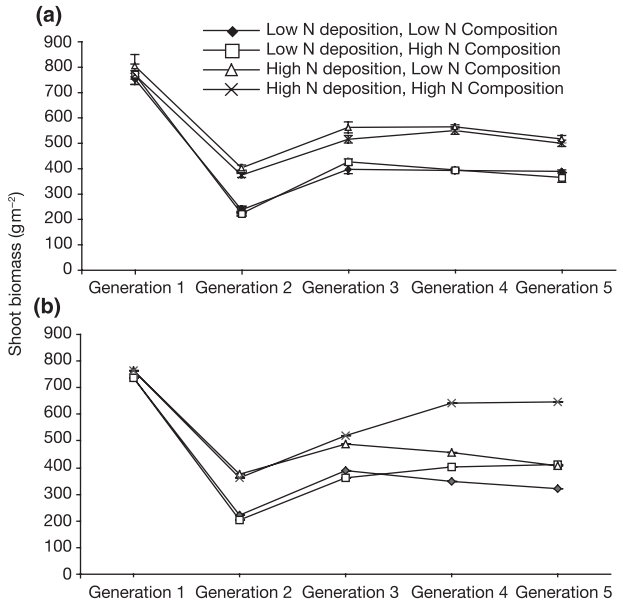

Figure 2 (a) Aboveground biomass for each treatment and generation and (b) predicted biomass in the absence of intraspecific frequency-dependent growth responses. Data presented in (a) are mean values (± SEM).

Evapotranspiration was significantly higher ($F_{1,12} = 25.3$, $P < 0.001$), and soil moisture and water through-flow significantly lower ($F_{1,12} = 29.7$, $P < 0.001$, $F_{1,12} = 37.3$, $P < 0.001$ respectively), in high N deposition treatments, while there were no effects of N composition ($F_{1,12} =$ 0.1–4.4, $P > 0.05$) Table 1, Fig. S3).

Hypotheses to explain the lack of compositional effects

Species differed in their aboveground biomass (species: $F = 59.1_{7,495}$, $P < 0.001$), at both levels of N deposition (species × N: $F = 2.5_{7,495}$, $P > 0.05$). Single degree of freedom contrasts found significant differences ($P < 0.05$) in the biomass of the dominants; *Solanum nigrum* produced more aboveground biomass than *Matricaria recutita* and *Sonchus asper* at both levels of N deposition. Hypothesis 1 was therefore not supported.

There was a positive relationship between plant biomass and response to N deposition which had a high overall significance ($r = 0.42$–0.68, $\chi^2 = 21.4$, d.f. $= 8$, $P < 0.01$), thus demonstrating that larger species were more frequent in the high N composition. Hypothesis 2 was therefore not supported.

There was much stronger support for the third hypothesis. *Sonchus asper*, which comprised 48% of all aboveground biomass harvested in the experiment over the last three generations, grew larger at lower frequency (frequency, $F_{1,30} = 171.0$, $P < 0.001$) especially in the high N deposition treatment (frequency × N deposition, $F_{1,30} =$ 9.7, $P < 0.01$). *Solanum nigrum*, also grew larger at lower

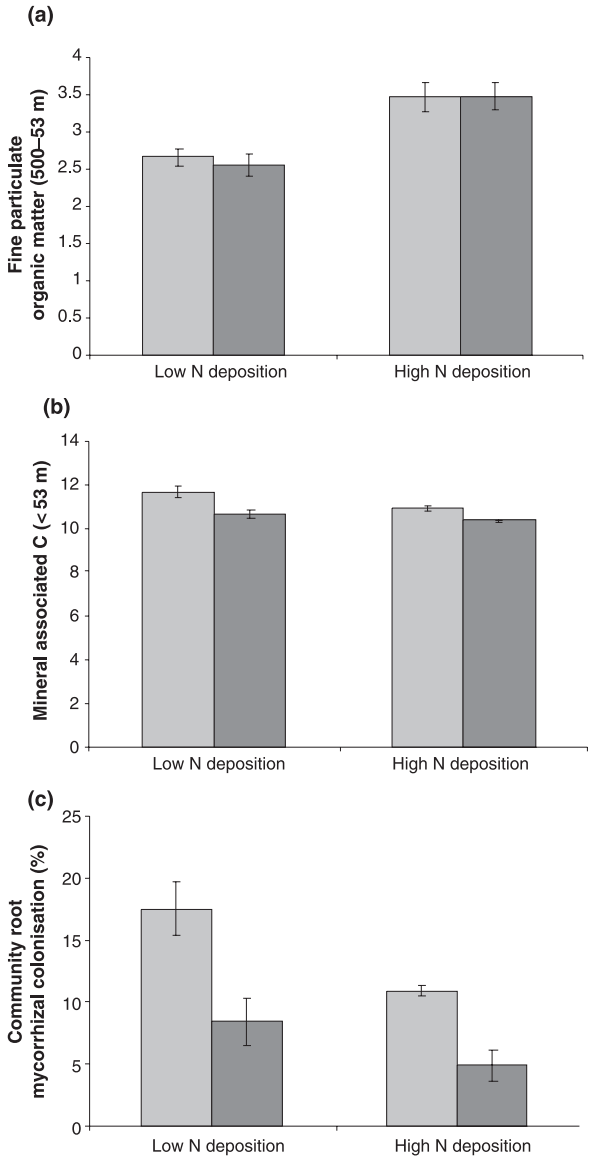

Figure 3 Treatment effects upon soil carbon fractions and mycorrhizal abundance at the end of the fifth generation. Lightly shaded bars, low N composition; heavily shaded bars, high N composition. Data presented are mean values (± SEM).

frequencies, but only in high N deposition microcosms (frequency, $F_{1,31} = 0.3$, $P > 0.05$, frequency × N deposition, $F_{1,31} = 12.4$, $P < 0.001$). In contrast, the biomass of *Matricaria recutita* was independent of frequency (frequency, $F_{1,30} = 0.0$, $P > 0.05$, frequency × N deposition, $F_{1,30} =$ 0.6, $P > 0.05$) (for statistical details see Table S4). These frequency-dependent changes in per capita performance led to a convergence in community biomass at each N deposition level; in the absence of this frequency dependence, the biomass in the later generations of the experiment would have shown a strong composition effect (Fig. 2).

DISCUSSION

Direct effects dominated ecosystem response to N deposition. These effects appeared to be driven by the stimulation of plant growth by N deposition and the elevated litter inputs that resulted from this productivity increase. Higher litter inputs account for higher soil C and N storage and the stimulation of decomposer activity and biomass in high N deposition microcosms. However, although increased litter inputs stimulated decomposer activity, this was not enough to prevent the accumulation of C and N in soil of the high N deposition treatment.

Higher plant biomass in high N deposition microcosms also explains higher evapotranspiration losses and corresponding declines in soil moisture content and water through-flow under elevated N deposition. In turn, these moisture differences may account for the failure of the decomposer community to respire all the increased litter inputs in the high N deposition treatment; soil moisture was reduced over the 5–25% range to which microbial activity is sensitive in sandy loam soils (Schjønning *et al.* 2003).

A notable result of our study was that mineral-associated C was lower under both high N deposition and high N composition. Whilst increased storage in the fine POM fraction under high N deposition probably reflects higher litter inputs we hypothesize that effects on mineral-associated C were mediated by AM fungi, which contribute to this fraction by producing glycoproteins (Rillig 2004). The composition effect on AM abundance might be attributed to the frequency of *Matricaria recutita*, while the N deposition effect may be explained by a reduced C allocation of plants to AM when N availability is raised (Treseder 2004). The impact of both direct and indirect (compositional) effects of N deposition on mineral-associated C, whether explained by AM fungi or not, raises questions about whether increased C inputs will translate into long-term C storage. It also adds to growing evidence (Neff *et al.* 2002; Mack *et al.* 2004) that a better understanding of soil C response to changing N availability is required to accurately predict ecosystem C dynamics.

With the exceptions discussed above, the species composition treatment had little effect on the ecosystem properties we measured. Poor support for hypotheses 1 and 2, and much stronger support for hypothesis 3 indicates that the general lack of compositional effects was not mediated by functional equivalence or a lack of correlation between a species response to N deposition and biomass production, but by frequency-dependent process acting at the population level. Although not exactly compensatory, frequency-dependent growth resulted in the dominant (*Sonchus asper*) producing a similar biomass within each level of the N deposition treatment, regardless of its initial density. The consequence of this was production of a fixed amount of community biomass at each N deposition level.

Although frequency dependence explained the lack of compositional effects it is conceivable that composition effects would have been stronger had the plant community been more functionally diverse. High N composition effects appear to have been observed where fertilization results in dominance shifts between functional groups in heathland (Van Vuuren *et al.* 1992) and tundra (Shaver *et al.* 2001) ecosystems. In Dutch heathlands, for instance, grasses (such as *Molinia caerulea*) outcompete and replace ericaceous dwarf shrubs (such as *Erica tetralix*) at higher DIN availabilities (Aerts & Berendse 1988) and so come to dominate areas of high N deposition. These compositional changes can result in increased productivity and acceleration of N mineralization rates (Van Vuuren *et al.* 1992) thus further enhancing the competitiveness of the grasses.

A strong composition effect on productivity may have been observed in our experiment if it had included legumes, which would be expected to be more frequent in low N compositions (Suding *et al.* 2005), and have the potential to generate a composition × N deposition interaction. This is because legumes access atmospheric N sources that would allow them to produce additional biomass in N limiting conditions. However, they may be less able to do this where N availability, and consequently light competition, is high. Stronger composition effects may also have emerged if communities had diverged more strongly, though this would be unlikely if frequency dependent growth continued to play an important role in controlling biomass production.

Conclusions from experimental microcosms should be extrapolated to field conditions with caution (Carpenter 1996). For example, some potential compositional effects of elevated N, such as regional extinctions, and switches between major plant functional groups, were not included in our experimental design. Nevertheless, our results demonstrate that ecosystem responses to N deposition were dominated by direct effects on plant growth and soil biochemistry rather than indirect effects mediated by changes to plant community composition. If similar results were obtained in natural ecosystems then this would suggest that the direct effects of global change on ecosystem function could be stronger than those of the widely studied effects of biodiversity loss (Hooper *et al.* 2005). While there is considerable evidence that dominance shifts between major functional groups will lead to major changes to ecosystem function (Jackson *et al.* 2002; Wardle & Zackrisson 2005) we suspect that most compositional change will probably have little effect on ecosystem function in comparison with the direct effects of factors causing this change (e.g. climate, disturbance and fertilization). Strong linkages between climatic and geological factors and ecosystem function, the scale invariance of relationships

between physical factors and ecosystem processes (e.g. Reich *et al.* 2006) and rapidly saturating relationships between biodiversity and ecosystem function (Hooper *et al.* 2005) all support this view. We need now to study the relative importance of these mechanisms under more natural field conditions, in functionally diverse communities, and at a variety of scales, in order to formulate predictive models of ecosystem response to global change.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available online from http://www.blackwell-synergy.com:

Appendix S1 Hypotheses for lack of composition effects.

Table S1 The relationship between aboveground vegetative biomass ($\log_e$ g) and seed output ($\log_e$ g).

Table S2 Comparisons of the estimated seed output (g m^{-2}) from the low N deposition/composition and high N deposition/composition chambers.

Table S3 Further statistical analysis of the main experimental variables, to Table S1.

Table S4 Tests of frequency dependence in per capita and population biomass for the three dominant species.

Figure S1 Biomass–fecundity relationships for the eight annual herb species used in the experiment.

Figure S2 Total soil carbon in the four treatments across the five generations. Data presented are means (± SEM).

Figure S3 The temporal pattern in soil moisture across the five generations.

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