GLOBAL BIOGEOCHEMICAL CYCLES, VOL. 25, GB2014, doi:10.1029/2010GB003869, 2011

Plant diversity effects on aboveground and belowground N pools in temperate grassland ecosystems: Development in the first 5 years after establishment

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Received 17 May 2010; revised 15 November 2010; accepted 16 February 2011; published 25 May 2011.

[1] Biodiversity is expected to improve ecosystem services, e.g., productivity or seepage water quality. The current view of plant diversity effects on element cycling is based on short-term grassland studies that discount possibly slow belowground feedbacks to aboveground diversity. Furthermore, these grasslands were established on formerly arable land associated with changes in soil properties, e.g., accumulation of organic matter. We hypothesize that the plant diversity-N cycle relationship changes with time since establishment. We assessed the relationship between plant diversity and (1) aboveground and soil N storage and (2) NO_3-N and NH_4-N availability in soil between 2003 and 2007 in the Jena Experiment, a grassland experiment established in 2002 in which the number of plant species varied from 1 to 60. The positive effect of plant diversity on aboveground N storage (mainly driven by biomass production) tended to increase through time. The initially negative correlation between plant diversity and soil NO_3-N availability disappeared after 2003. In 2006 and 2007, a positive correlation between plant diversity and soil NH_4-N availability appeared which coincided with a positive correlation between plant diversity and N mineralized from total N accumulated in soil. We conclude that the plant diversity-N cycle relationship in newly established grasslands changes with time because of accumulation of organic matter in soil associated with the establishment. While a positive relationship between plant diversity and soil N storage improves soil fertility and reduces fertilizing needs, increasingly closed N cycling with increasing plant diversity as illustrated by decreased NO_3-N concentrations in diverse mixtures reduces the negative impact of agricultural N leaching on groundwater resources.

Citation: Oelmann, Y., et al. (2011), Plant diversity effects on aboveground and belowground N pools in temperate grassland ecosystems: Development in the first 5 years after establishment, *Global Biogeochem. Cycles*, *25*, GB2014, doi:10.1029/2010GB003869.

1. Introduction

[2] The global extinction of species has raised concern about the effect of biodiversity on ecosystem functioning and the resulting services for mankind [*Schulze and Mooney*, 1993]. As a consequence of intensified management, species loss in managed grasslands has been observed during the last decades [*Minns et al.*, 2001] and natural grasslands have nearly completely disappeared [*Sterling and Ducharne*, 2008]. Species richness in grasslands ranges from very low in highly productive monocultures or two-species mixtures used for agriculture to very high in extensively used meadows. This range facilitates experimental studies on the relationship between plant diversity and ecosystem functioning (single experiments cited in reviews by *Hooper et al.*

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0886-6236/11/2010GB003869

Table 1. Weather Conditions During the Study Period[a]

		ST (° C)			SM (g kg^{-1})		Phenological Stage	
	Mean *T* (° C)	Spring	Fall	Rainfall Sum (mm)	Spring	Fall	Spring	Fall
LTM	9.3 a			587 a				
2003	9.9 a	4.4 c	12. 5 c	436 b	331 b	175 d	8	4
2004	9.4 a	2.5 d	12.3 c	573 a	285 d	220 c	5	4
2005	9.2 a	8.4 a	14.2 a	422 b	272 e	144 e	nd	6
2006	10.0 a	2.8 d	13.2 b	493 a	303 c	295 b	5	4
2007	10.2 a	6.1 b	12.3 c	710 a	371 a	344 a	5	nd

[a] Mean temperatures (*T*) and rainfall sums refer to yearly data, whereas soil temperature (ST) and soil moisture (SM) were calculated for the 14 day period before sampling in spring and fall, respectively. Different letters indicate significant differences between the respective year and the long-term mean (LTM; mean *T*, rainfall sum) or among years (ST, SM); that is, values followed by an "a" are significantly greater than values followed by "b," etc. The phenological stage refers to the median of all monocultures before the harvest with a scale ranging from 1 to 15 (1, seedling; 15, disappearing after senescence); nd, not determined.

[2005] and *Hillebrand and Matthiessen* [2009]). In these studies, biodiversity was considered as the driven variable depending on, e.g., land-use intensity or amount of added fertilizer. However, recent experimental research has demonstrated that biodiversity is also a driver of ecosystem services if biodiversity varies under the same type of land management [*Loreau et al.*, 2002; *Hooper et al.*, 2005; *Weigelt et al.*, 2009]. Plant diversity had positive effects on productivity and seepage quality [*Hector et al.*, 1999; *Scherer-Lorenzen et al.*, 2003; *Hooper et al.*, 2005].

[3] The underlying mechanistic hypothesis is that plants can use resources in a complementary way such that strong competition in diverse systems promotes niche differentiation in space and time, resulting in an increased community resource use compared with less diverse systems [*Hooper et al.*, 2005]. Because N is the quantitatively most important plant nutrient which also has environmental relevance as a potential pollutant, most plant diversity studies have addressed the effects of a loss in plant diversity on N pools [*Tilman et al.*, 1996; *Hooper and Vitousek*, 1997; *Niklaus et al.*, 2001; *Scherer-Lorenzen et al.*, 2003].

[4] Plant diversity increased aboveground biomass production and thus, aboveground N storage [*Oelmann et al.*, 2007b]. As a result, NO_3-N availability in soil decreased with increasing plant diversity [*Niklaus et al.*, 2001; *Scherer-Lorenzen et al.*, 2003; *Oelmann et al.*, 2007b]. Gross or net N mineralization and thus N supply to plants, were positively related to plant diversity contributing to explain the finding of increased N storage in aboveground plant material [*Zak et al.*, 2003; *Dybzinski et al.*, 2008; *Fornara et al.*, 2009]. The amount of N released per unit of time among other factors (pH, soil moisture, substrate quality, composition of decomposing organisms) depends on the total storage of N in soil. *Fornara and Tilman* [2008] showed that highly diverse mixtures stored more total N in soil than monocultures after 12 years. As a consequence, the amount of N released and N_{min} availability (NH_4-N + NO_3-N) in soil likely also changed with time after establishment of the grassland studied by *Fornara and Tilman* [2008]. Thus, one might expect counterbalancing effects of increased N release in soil and increased N uptake by plants in highly diverse mixtures. Depending on the relation among the differently directed processes, the relationship between plant diversity and N_{min} availability in soil might either be positive, negative, or missing. However, up to now no longer-term observations of N_{min} availability in soil under differently diverse grassland communities are available.

[5] Finally, it remains unclear whether the temporal course of plant diversity effects on belowground processes feeds back on N storage in aboveground biomass. Former studies on productivity suggest that the effect of plant diversity on aboveground N storage persists through time with an either saturating or increasing slope of the regression if constant N concentrations in aboveground biomass are assumed [*Cardinale et al.*, 2007; *van Ruijven and Berendse*, 2009]. Functional plant groups strongly influence aboveground and belowground N cycling, e.g., through the ability to fix atmospheric N_2 (legumes [*Mulder et al.*, 2002; *Spehn et al.*, 2002]) or to exploit N in soil more exhaustively than other functional groups because of the extensive rooting system (grasses [*Craine et al.*, 2002]). Therefore, it is essential to include functional groups in the analyses of plant diversity effects on aboveground and belowground N pools.

[6] Our objectives were to assess longer-term trends (5 years) of the relationship between plant diversity and aboveground biomass and belowground soil N storage and between plant diversity and NH_4-N and NO_3-N availability in soil, and to elucidate possible mechanisms underlying these temporal trends. Special emphasis was paid to the effect of functional plant groups (legumes, grasses, tall and small herbs). As grasslands are widespread and the conversion of arable land to grassland is common in the temperate zone [*Soussana et al.*, 2004], our results might contribute to answer the question if the maintenance of plant diversity should be considered as a beneficial element of agricultural practice.

2. Materials and Methods

2.1. Study Site

[7] The field site is located close to the German city of Jena. Mean annual air temperature is 9.3°C, and mean annual precipitation amounts to 587 mm (1961–1990) [*Kluge and Müller-Westermeier*, 2000]. Weather conditions during the study period and compared to the long-term mean (LTM) are given in Table 1. The soil is a Eutric Fluvisol developed from up to 2-m-thick fluvial sediments that are almost free of stones. Due to fluvial dynamics, the texture ranges from sandy loam near the river to silty clay with increasing distance from the river. The plots were

arranged in four blocks parallel to the river on texturally homogeneous subareas and the block effect is included in the statistical analyses. Initial organic C concentrations ranged from 13 to 33 g kg^{-1}, organic C:N ratios from 8 to 15, and pH (H_2O) from 7.1 to 8.4. The soil contains some carbonates (15 g kg^{-1} CO_3^{2-}-C). The site was used as an arable field for the last 40 years prior to the experiment.

[8] The experimental design is described by *Roscher et al.* [2004]. The main experiment comprises 82 plots (20 × 20 m, established from seeds in May 2002) with different levels of species richness (1, 2, 4, 8, 16, 60), and different numbers (1, 2, 3, 4) of functional groups (grasses, small herbs, tall herbs, legumes). Plant species were chosen by the random replacement method from a species pool of 60 species from the Molinio-Arrhenatheretea meadows, Arrhenatherion community [*Ellenberg*, 1996]. Each level of species richness is replicated four times per block (n = 16, 16, and 60 species mixtures: n = 14 and n = 4, respectively). The number of sown species strongly correlated with the number of established target species ($r > 0.95$ in all years) and verified the successful establishment of the species richness gradient [*Marquard et al.*, 2009]. The management of all plots was adapted to extensive meadows used for hay production and mown twice a year in June and September. Plots were not fertilized during the experimental period. To maintain the sown species diversity level, plots were weeded twice a year by cutting the weeds aboveground. Therefore, soil disturbance was minimized and did not differ among diversity levels.

2.2. Sampling

[9] Each spring and fall from 2003 to 2007, phenology, i.e., plants development stage, was recorded according to a 15-part ordinal scale covering the vegetative stage and different stages of inflorescence and fruit development. Phenology was estimated as an average value per plot in monocultures of all species shortly before harvesting aboveground biomass. In spring and fall from 2003 to 2007 after plants started flowering (Table 1), aboveground plant biomass was harvested on all plots within a frame (0.2 × 0.5 m, height 0.03 m) at four randomly located sites per plot. Plant biomass of mixtures was separated into species for all harvests (except August 2004: samples sorted into legume and nonlegume species). After oven-drying (70°C) to constant weight, plant material collected from each of the four randomly located sites within each plot was weighed.

[10] In spring and fall 2003–2007, five soil cores (diameter 0.01 m) of a depth of 0–0.15 m of the mineral soil from each of the experimental plots were pooled to one composite sample per plot. The determination of changes in total N storage in soil was based on two additional sampling campaigns. In April 2002, 2004, and 2006, stratified soil sampling was performed to a depth of 30 cm. Independent samples (2002: n = 5; 2004 and 2006 n = 3) per plot were collected using a split tube sampler with an inner diameter of 4.8cm (Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands). Soil samples were dried at 40°C and segmented into six 5 cm depth subsamples per core [*Steinbeiss et al.*, 2008].

[11] We conducted an incubation experiment with soil and plant material to determine net mineralization rates. Ten soil cores (depth 0–15 cm, diameter 2 cm) per plot were sampled in November 2006 and pooled to one composite sample per plot. Soil was sieved to < 2 mm, visible roots were removed. Plant material of each plant species was sampled in the "common garden" where all plants of the 60-species pool were grown on small plots (1 m^2) adjacent to the main experimental plots. Additionally, plant material of all of the four 60-species mixtures was harvested on an area of 0.1 m^2 each. Plant material was chopped with scissors. Soil samples and plant material were kept at 4°C until the incubation started.

2.3. Extractions and Chemical Analyses

[12] To determine N concentrations in aboveground biomass, living plant material from all samples per plot was pooled together per harvest campaign and ground with a Cyclotec 1093 Sample Mill (Foss Tecator, Hoganas, Sweden). Twenty mg of the ground plant material was analyzed for plant N concentration with an elemental analyzer CE 1110 (Carlo Erba Instruments, Milan, Italy). Aboveground N pools were then calculated using aboveground biomass and N concentrations. Total N concentrations in soil were determined with the same elemental analyzer as plant N concentrations.

[13] As an estimate of plant-available N, soil inorganic N (NO_3-N and NH_4-N, the sum of which is termed mineral N or N_{min}) concentrations were determined by extraction of soil samples with 1 M KCl solution [*Mulvaney*, 1996]. Nitrate-N and NH_4-N concentrations were measured in the soil extract with a Continuous Flow Analyzer (CFA, 2003–2005: Skalar, Breda, Netherlands; 2006–2007: AutoAnalyzer, Seal, Burgess Hill, United Kingdom). Availability of NO_3-N and NH_4-N (N_{min} = sum of NO_3-N and NH_4-N) in soil was calculated based on soil densities averaged across blocks. In fall 2006, our estimate of NH_4-N availability in soil is based on a reduced data set (n = 53), because we had to exclude samples stored in a fridge that did not work properly (temporally, $T > 4°C$). Nevertheless, the experimental design covered by the reduced set of samples was still balanced with respect to plant diversity ($8 < n < 12$ for each plant diversity level, except for the 60-species mixture with n = 4). Although we are aware of the low temporal resolution, former results showed no difference in diversity effects between soil sampling twice per year and continuous vacuum extraction of soil solution with biweekly sampling intervals during the first 2 years of The Jena Experiment [*Oelmann et al.*, 2007b], suggesting that our determination of mineral N concentrations in soil is representative for the winter period (spring sampling) and the vegetation period (fall sampling).

[14] To determine N release rates, we used an incubation approach described by *Nadelhoffer* [1990]. Field-fresh soil and moist plant material were incubated in a bench-top filter unit for 66 days at 20 ± 1°C. We used the seeded contribution of a plant species to a given mixture as the proportional weight of the 10 g moist plant material. For 60-species mixtures, 10 g plant material harvested in the respective plot in the field was added to the soil. We applied 0.1 l of a nutrient solution (not containing N) according to *Nadelhoffer* [1990]. We leached the samples with 0.1 l of the nutrient solution by applying a vacuum of 30 kPa once a week. In the leachates, total dissolved nitrogen (TDN) concentrations were measured

with a Continuous Flow Analyzer (AutoAnalyzer, Seal, Burgess Hill, United Kingdom).

2.4. Calculations and Statistical Analyses

[15] We calculated net N release by mineralization based on the change of total N storage in soil. The change of total N storage in soil was related to plant diversity [*Steinbeiss et al.*, 2008] which reflects the accumulation of total N in soil and is coupled to the simultaneous increase in soil organic matter stocks associated with the change from organic-matter depleted arable soil to grassland. We assumed that N release by mineralization of this accumulated N in soil would also be related to plant diversity and thus, might influence the relationship between plant diversity and availability of N_{min} in soil. We calculated the net N release from accumulation of total N in soil (2002–2006) per plot (NR_i in g m^{-2} with i describing the individual plot). In the calculation of the accumulation of total N in soil, we had to account for compaction of soil because of the land-use change (cessation of ploughing, weeding). Assuming conservation of mass, we calculated the thickness of the sampled cores in 2002 based on the differences of soil densities (2002 and 2006) per depth increments (0–5 cm, 5–10 cm, 10–15 cm) for each Block. For 2002 and 2006, soil mass was calculated as the product of soil volume and soil density [*Steinbeiss et al.*, 2008]. We multiplied soil mass and N concentrations per depth increment and summed N storage in soil for a depth of 0–15 cm for 2002 and 2006. The accumulation of total N storage in soil per plot i (ΔNS_i in g m^{-2}) from 2002 to 2006 was calculated as the difference in total N storage in soil between the respective years. Five outliers (smaller or greater than twofold standard deviation) were excluded from further analyses.

[16] We divided the cumulative mass of TDN in the leachates collected from the incubated soil cores, i.e., the sum of the mass of TDN of all percolation dates, by the initial N storage in the incubated soil monoliths. We used this ratio as N release rates per plot i (NRR_i, in mg N [g N]$^{-1}$ d^{-1}). We used correction factors, i.e., $g\theta$ (correction factor based on soil moisture) and fT (correction factor based on soil temperature; *Rodrigo et al.*, 1997), for N release rates under field conditions. We calculated depth-weighted mean (DWM) soil moisture (8 and 16 cm soil depth) based on the automatic records of the central climate station at the field site. Soil moisture of this location represents the mean of the study site (within mean ± 1/2 standard deviation of soil moisture of all plots determined gravimetrically for the 10 N_{min} sampling campaigns). Mean soil temperature was automatically recorded at every plot in a depth of 15 cm. We averaged soil temperatures and DWM soil moistures per day. To calculate $g\theta$, we assumed that microbial activity was linearly related to soil moisture and used the incubation release rates assessed for 31.71 Vol.% water content and zero water content in case of complete dryness associated with no microbial activity as references. Temperature effects were accounted for by using a Q_{10} value of 2 according to the Van't Hoff function [*Rodrigo et al.*, 1997]. If the mean daily temperature was less than 0°C, microbial activity was set to zero. Both correction factors were multiplied to yield relative rates of microbial activity [*Rodrigo et al.*, 1997]. The net N release of accumulation of total N in soil of each plot was calculated according to equation (1), with t_0 = 01/01/2003 (first date with both soil moisture and soil temperature available) and t_1 = 31/03/2006 (date of sampling of N storage in soil).

$$NR_i = \Delta NS_i \times NRR_i \times 1000^{-1} \times \sum_{t0}^{t1} g\theta \times fT \qquad (1)$$

During the first 6 months after establishment of the experiment, no simultaneous measurements of soil moisture and temperature were available. By excluding this period we underestimated the sum of N mineralized from total N accumulated in soil. However, we can assume that incorporation of organic material in soil [*van Ruijven and Berendse*, 2009] and related N release rates were small compared to the following years which were characterized by an increasing establishment of the microbial community in soil [*Eisenhauer et al.*, 2010].

[17] All data were log transformed before statistical analyses to meet requirements of analyses of variance. To assess differences in temperature and rainfall, we used the nonparametric Wilcoxon matched pairs test for the monthly data as a conservative approach. In case of the 14 day period before sampling, we tested differences in soil temperature and soil moisture by means of the nonparametric Friedman test including the Bonferroni correction because of multiple testing. We used an ANOVA (Type I, hierarchical approach) to disentangle effects of Block (random factor), species richness and the presence or absence of functional plant groups in mixture (fixed factors). We excluded functional group richness in our statistical model because we already showed that plant species richness was the main explanatory variable for N storage in aboveground biomass and N availability in soil [*Oelmann et al.*, 2007b]. Post hoc tests (Games Howell) were used to elucidate differences among the factors and among years. We applied a repeated measures ANOVA including the same statistical model as described above to elucidate the effect of time on the factors of the model. We used the Greenhouse-Geisser adjustment for within-subject effects and the differences between subsequent years for the test of within-subject contrasts. To characterize the relationship between plant diversity and aboveground or belowground N storage or N_{min} availability in soil in more detail, we calculated a linear regression of these response variables (means per diversity level) on plant diversity. The SPSS software package was used for all statistical analyses (SPSS 15.0, SPSS Inc., Chicago, Illinois, United States).

3. Results

[18] Phenological results of all monocultures were in a narrow range, i.e., plants started flowering (Table 1). If considering particular functional plant groups, small herbs had fully developed flowers before spring harvests, whereas legumes and tall herbs started fruiting before the second harvest (data not shown). As compared to the other years, aboveground N storage was significantly lower in spring 2005 with lowest soil moisture before sampling and the lowest yearly rainfall sum in 2005 (Table 1 and Figure 1). In fall 2003, plant mixtures consistently stored significantly more N aboveground than in the other fall campaigns despite the lowest soil moisture conditions before sampling (Table 1 and Figure 1). Plant diversity was positively related

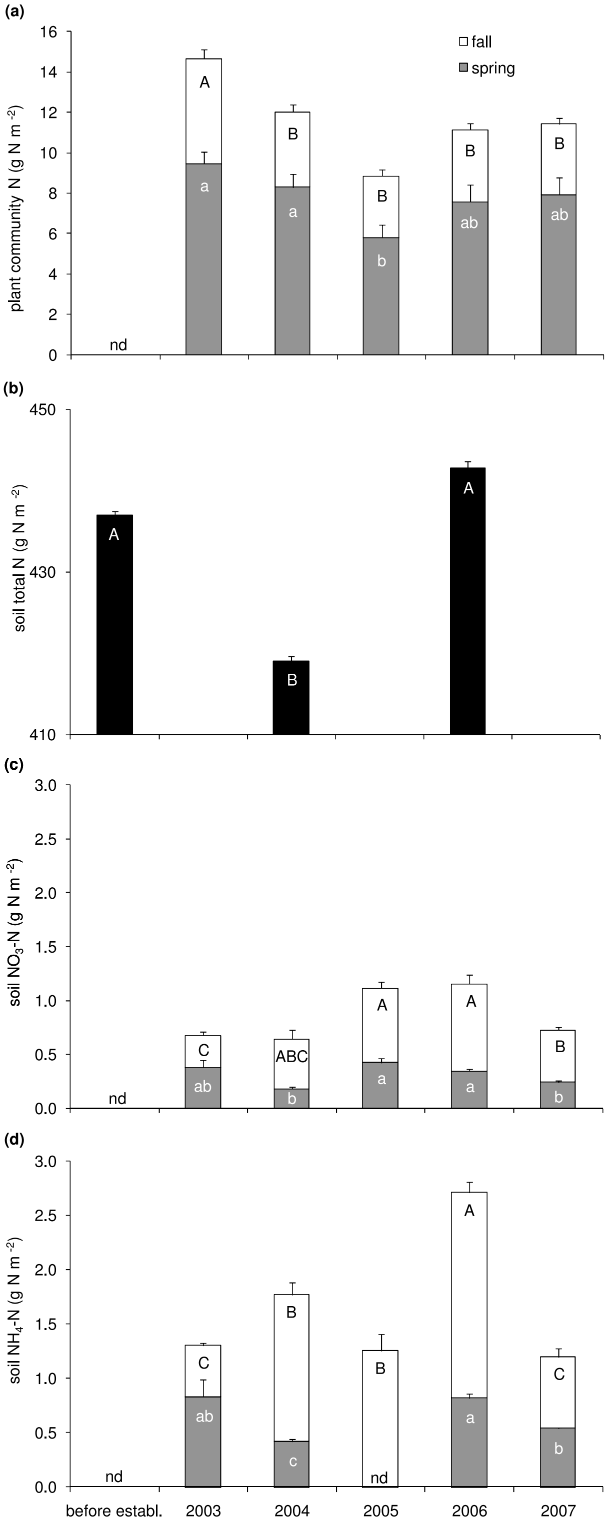

Figure 1. Temporal course of (a) plant community N storage, (b) soil total N storage, (c) soil NO$_3$-N availability, and (d) soil NH$_4$-N availability. Note that data on soil NO$_3$-N and NH$_4$-N availability reflect one-point-in-time measurements. Grey and white bars refer to the spring and fall sampling campaigns, respectively. Different letters indicate significant differences among years. Means and standard errors are given; nd, no data available.

to aboveground N storage throughout the experiment (Figure 2 and Table 2) and explained, on average, 18% of the variance (range 7 to 31%). One exception of this otherwise consistent trend was spring 2004, where 2- and 60-species mixtures significantly differed from all other plant diversity levels ($p < 0.01$) without a linear trend (Figure 2). In spring, the effect of plant diversity changed with time as indicated by significant differences between subsequent years from 2003 to 2006 (repeated measures ANOVA, within-subject effects, $2.0 < F < 4.0$, $0.001 < p < 0.048$). This temporal trend was characterized by increasing slopes of the regression between plant diversity and aboveground N storage toward the end of the observation period (Figure 2). Neither air temperature nor rainfall could explain the temporal trend as both were not significantly related to the slopes of the regression ($0.13 < r < 0.60$, $0.09 < p < 0.73$).

[19] Mixtures containing legumes had significantly higher aboveground N storage. On average of all sampling campaigns legume-containing mixtures stored 237% more aboveground N than mixtures without legumes. The presence or absence of legumes in mixtures explained the greatest proportion of variance in the statistical model (29 ± standard error [SE] 3%). With proceeding grassland establishment the surplus of aboveground N storage in mixtures containing legumes compared to mixtures without legumes increased for the spring sampling campaigns (2003: 86%, 2004: 129%, 2005: 287%, 2006: 445%, 2007: 357%) whereas there were no temporal trends in fall. The presence of small herbs had a significant effect on aboveground N storage (Table 2). However, there was no consistent variation of aboveground N storage if small herbs were present; the differences between mixtures with and without small herbs ranged between −27% and +18% without a temporal trend. These effects remained even if the worst performing monoculture of small herbs, *Bellis perennis*, which frequently yielded no observable aboveground biomass production, was excluded from the statistical analyses (data not shown).

[20] Soil total N storage in the 0–15 cm soil layer was lower in 2004 than in 2002 and 2006 (Figure 1). Plant diversity did not affect N storage in soil ($1.2 < F < 1.9$; $0.100 < p < 0.337$; data not shown). We found no effect of functional plant groups ($0.0 < F < 3.4$; $0.070 < p < 0.964$) with the exception of a significantly reduced N storage in soil if small herbs were present ($F = 4.6$; $p = 0.036$; data not shown). The accumulation of total N storage in soil from 2002 to 2006 ranged from −11.4 to 15.2 g N m^{-2} yr^{-1} in 0 to 15 cm soil depth.

[21] Nitrate-N availability in soil significantly differed among years without a temporal trend (Figure 1). In spring 2004, low temperatures (Table 1; 8 out of 14 days, $T < 0°C$) were related to low NO$_3$-N availability in soil. Contrarily, high soil temperatures coincided with high NO$_3$-N availability in soil in 2005 and 2006 (Table 1 and Figure 1). There was a significant regression of NO$_3$-N availability in soil on plant diversity during the first two sampling campaigns (Figure 2b, spring and fall 2003) which on average explained 13% of the variance (range 9 to 19%). The slope of the regression of NO_3^- availability in soil on plant diversity was negative (Figure 2b). The relationship between plant diversity and NO$_3$-N availability in soil only existed

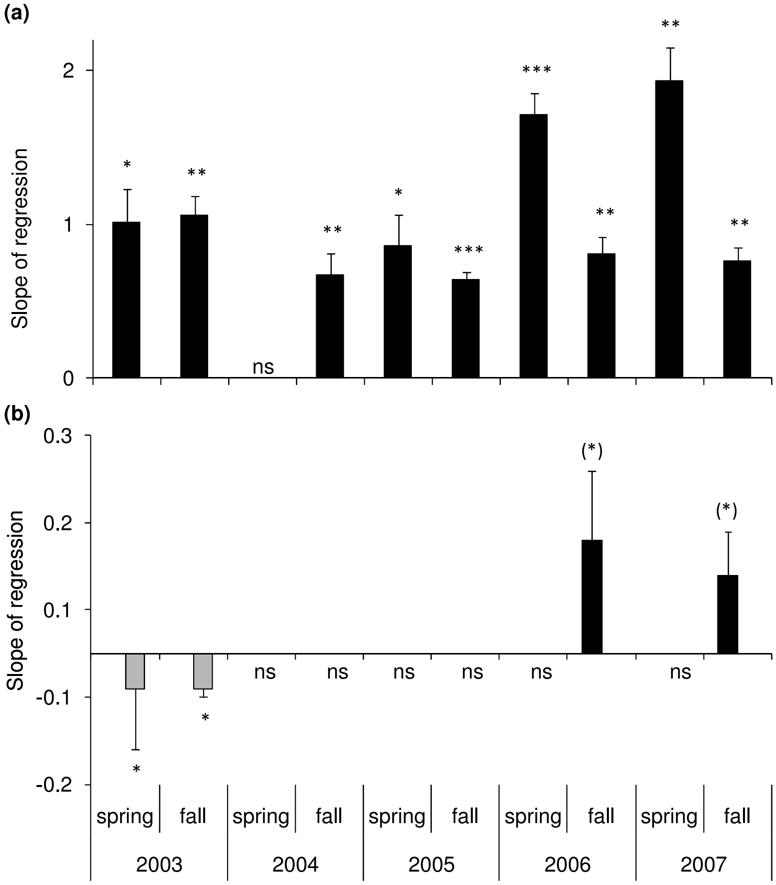

Figure 2. Slopes of the significant regressions between plant diversity ($\log_2$) and (a) aboveground N storage or (b, gray bars) NO_3-N availability in soil and (b, black bars) NH_4-N availability in soil from 2003 to 2007. Error bars depict the standard error of the slope. In case of significant relationships for NO_3-N, there was no significant relationship for NH_4-N and vice versa. Note the reduced data set ($n = 53$) in fall 2006; ns, not significant; (*), $p < 0.1$, marginally nonsignificant; *, $p < 0.05$; **, $p < 0.001$; ***, $p < 0.0001$.

during the first two sampling campaigns and thus, changed through time (significant effect of time in Table 3).

[22] In fall, the presence of legumes increased NO_3-N availability in soil by 65 ± SE 14% compared to mixtures without legumes (Table 3). In spring, this increase was less pronounced (30 ± SE 14%). The presence of legumes in mixtures contributed 16 ± SE 2%, on average, to the explanation of the variance of NO_3-N availability in soil which was greater than the contribution of any other factor in the statistical model. The effect of presence of legumes did not vary with time (Table 3). Similarly to aboveground N storage, we found a significant negative effect of the presence of small herbs on NO_3-N availability in soil (Table 3) which ranged between −18% and −54% comparing mixtures without and with small herbs.

[23] Ammonium-N availability in soil significantly differed among years without a temporal trend (Figure 1). Similar to NO_3-N, NH_4-N availability in soil was significantly reduced compared to the other sampling campaigns when mean temperatures were low (Table 1 and Figure 1). Block had a significant effect on NH_4-N availability in soil (Table 4). Sometimes Block 1 had significantly higher NH_4-N availability in soil than the other blocks (Games Howell; $< 0.0001 < p < 0.025$), sometimes this was the case for Block 4 (Games Howell; $p < 0.0001$). Plant diversity was significantly related with NH_4-N availability in soil in fall 2006 and 2007 (Figure 2 and Table 4) explaining 6 to 7% of the variance in NH_4-N availability in soil. No other factor in the statistical model had a consistently (i.e., occurred in more than one sampling campaign) significant effect on NH_4^+ availability in soil.

[24] Nitrogen release rates were determined for each individual plot by incubation in the laboratory and ranged from 0.09 to 1.44 mg N $(\text{g N})^{-1}$ d^{-1} (data not shown). Combined with the accumulation of total N in soil between 2002 and 2006 (also available for each individual plot), and considering the mean temporal development of soil moisture at the whole field, the range of N mineralized from total N accumulated in soil was between −17.7 and 19.3 g N m^{-2} $(4\ \text{yr})^{-1}$. This estimate includes an uncertainty mainly arising from the fact that we had to use one mean water content course for the whole experimental area. As the mean deviation of the water contents in the individual plots (gravimetrically determined twice a year) was 7% of the centrally registered mean water content of the whole experimental area, our estimate of mineralized N has a similar

Table 2. ANOVA (Type I) Results of Aboveground N Storage From 2003 to 2007[a]

	2003	2004	2005	2006	2007	Time
			Spring			
Block	0.82	7.69**	1.53	2.83*	2.63	1.35
SR	4.31* ↑	2.59* ↑	4.83** ↑	8.80*** ↑	12.92*** ↑	3.29*** ↑
LEG	16.82** ↑	42.24*** ↑	83.13*** ↑	97.08*** ↑	94.81*** ↑	12.72*** ↑
GR	0.38	0.18	1.5	2.33	0.16	0.63
tH	1.50	5.17* ↑	2.89	0.05	0.09	1.95
sH	4.53* ↓	19.00*** ↓	19.63*** ↓	7.68** ↓	10.61* ↓	1.25
SR × LEG	0.61	1.01	2.58*	2.66*	0.15	1.55
SR × tH	na	0.92	na	na	na	na
SR × sH	0.50	1.22	1.10	0.77	2.72*	na
			Fall			
Block	1.03	0.29	0.22	3.03*	3.42*	2.60*
SR	5.25** ↑	3.72* ↑	4.47* ↑	7.81*** ↑	11.22*** ↑	0.67
LEG	14.19** ↑	64.09*** ↑	60.21*** ↑	46.29*** ↑	42.19*** ↑	4.78* ↑
GR	0.51	0.17	0.44	0.69	0.74	1.24
tH	3.70	0.22	0.18	0.03	1.04	1.17
sH	12.94** ↓	11.00* ↓	10.79* ↓	17.56*** ↓	4.39* ↓	1.85
SR × LEG	1.13	0.38	2.53*	2.00	1.45	na
SR × sH	0.43	0.69	0.50	1.48	0.57	na

[a] Interactions between species richness (SR) and the respective functional plant groups (LEG, GR, tH, and sH represent presence of legumes, grasses, tall herbs, and small herbs, respectively) were included in the model if effects of SR or functional groups were significant. Time refers to the interaction term testing the within-subject effects in a repeated measures ANOVA (Type I). *F* values are given in bold if $p < 0.05$. Symbols used are as follows: *, $p < 0.05$; **, $p < 0.001$; ***, $p < 0.0001$; na, not applicable. Arrows indicate positive (↑) or negative (↓) effects of factors.

uncertainty. Furthermore, we used published transfer functions of climatic conditions to mineralization rates that were developed for other study sites than ours possibly resulting in an additional, unquantifiable bias. Significantly more N was mineralized from total N accumulated in soil in mixtures with higher plant diversity (eight-species mixtures > two- and four-species mixtures; Least Squares Differences, $0.04 < p < 0.05$, Figure 3). The presence of legumes significantly increased the N mineralized from total N accumulated in soil (ANOVA, Type I, statistical model see Tables 2 to 4; $F = 12.7$, $p = 0.001$, explained proportion of sum of squares [SS%] = 11%). The absolute difference in N mineralized from total N accumulated in soil between 60-species mixtures and monocultures was 3.1 g N m^{-2} $(4\ yr)^{-1}$ in the soil depth layer of 0–15 cm. Assuming a linear function (through time) of N accumulation in soil and

Table 3. ANOVA (Type I) Results of NO_3-N Availability in Soil (0–15 cm) From 2003 to 2007[a]

	2003	2004	2005	2006	2007	Time
			Spring			
Block	0.60	4.28*	7.25**	1.10	8.90***	2.02
SR	3.26* ↓	2.03	0.43	2.10	2.06	2.56*
LEG	0.77	17.49** ↑	0.49	14.35** ↑	0.22	1.28
GR	0.69	6.70* ↓	1.25	0.24	1.64	1.18
tH	0.08	0.05	0.00	0.18	6.01	0.07
sH	0.43	7.25* ↓	0.22	5.54* ↓	1.85	0.89
SR × LEG	na	0.53	na	na	na	na
SR × GR	na	0.26	na	na	na	na
SR × sH	na	0.4	na	na	na	na
			Fall			
Block	1.55	1.52	0.18	1.30	0.57	0.97
SR	3.44* ↓	0.57	1.46	1.37	1.12	0.75
LEG	43.94*** ↑	10.07* ↑	17.34*** ↑	1.48	10.50* ↑	1.37
GR	8.89* ↓	0.05	1.08	1.66	0.24	1.73
tH	0.02	4.28* ↓	7.21* ↓	0.32	1.10	2.36
sH	8.09* ↓	4.18* ↓	6.55* ↓	0.03	2.39	1.42
SR × LEG	1.94	na	na	na	na	na
SR × GR	1.26	na	na	na	na	na
SR × sH	0.34	na	na	na	na	na

[a] Interactions between species richness (SR) and the respective functional plant groups (LEG, GR, tH, and sH represent presence of legumes, grasses, tall herbs, and small herbs, respectively) were included in the model if effects of SR or functional groups were significant. Note the reduced data set ($n = 53$) in fall 2006 (see section 2). Time refers to the interaction term testing the within-subject effects in a repeated measures ANOVA (Type I). *F* values are given in bold if $p < 0.05$. Symbols are as follows: *, $p < 0.05$; **, $p < 0.001$; ***, $p < 0.0001$; na, not applicable. Arrows indicate positive (↑) or negative (↓) effects of factors.

Table 4. ANOVA (Type I) Results of NH_4-N Availability in Soil (0–15 cm) From 2003 to 2007[a]

	2003	2004	2005	2006	2007	Time
			Spring			
Block	0.88	23.77***	nd	5.62*	1.06	4.04*
SR	2.27	0.51	nd	0.23	1.34	2.04
LEG	0.92	0.03	nd	0.50	0.03	2.04
GR	0.04	1.72	nd	1.04	0.60	0.18
tH	0.76	0.11	nd	0.07	0.95	1.2
sH	0.00	0.47	nd	1.48	2.31	0.13
			Fall			
Block	30.56***	5.08*	2.41	0.49	2.62	1.40
SR	1.12	0.40	0.45	1.73[b] ↑	2.41* ↑	0.93
LEG	0.16	0.47	0.38	1.62	1.11	0.84
GR	1.29	1.21	0.03	0.97	0.09	0.45
tH	1.77	0.13	0.30	0.08	0.69	0.28
sH	1.15	0.67	9.33* ↑	0.33	0.69	1.71

[a] Time refers to the interaction term testing the within-subject effects in a repeated measures ANOVA (Type I). There were no concomitantly significant effects of species richness (SR) and functional groups (LEG, GR, tH, and sH represent presence of legumes, grasses, tall herbs, and small herbs, respectively); therefore, interactions were not included in the model. *F* values are given in bold if $p < 0.05$. Symbols are as follows: *, $p < 0.05$; **, $p < 0.001$; ***, $p < 0.0001$; na, not applicable; nd, no data available. Arrows indicate positive (↑) or negative (↓) effects of factors.

[b] The 60-species mixtures are significantly different from the 4-species and 8-species mixtures.

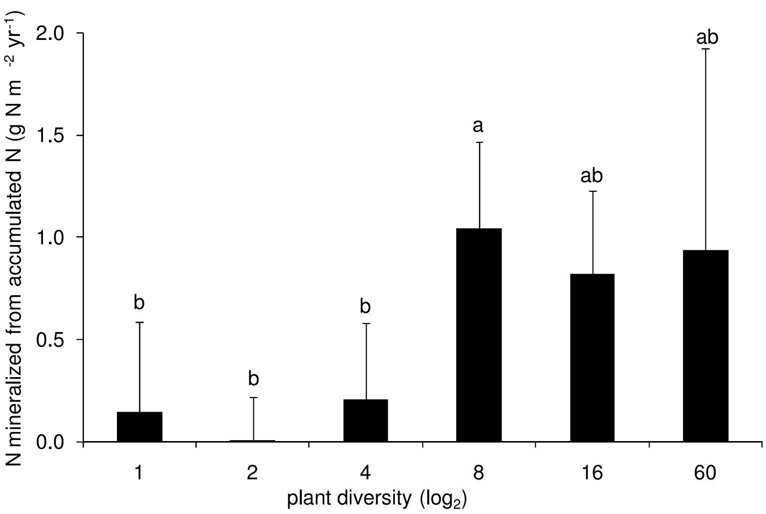

Figure 3. Relationship between plant diversity and N mineralized from total N accumulated in soil in 0 to 15 cm soil depth. Different letters indicate significant differences among diversity levels. Means and standard errors are given.

30 to 50% contribution of NO_3-N to N mineralized from total N accumulated in soil (mean range of the contribution of NO_3-N to N_{min} from 2003 to 2007), monocultures would have differed in the NO_3-N released by mineralization from total N accumulated in soil by 0.2 to 0.4 g N m^{-2} from 60-species mixtures in spring 2003.

4. Discussion

[25] In all monocultures, plant started flowering before the harvest (Table 1) indicating that weather conditions of the respective study year had only minor effects on phenology and plant communities were comparable at the time of harvesting. Two of the study years were exceptionally dry (Table 1). In spring 2003, productivity (and aboveground N storage) at the field site was significantly higher than in the other years. This is contrary to reduced productivity caused by the heat and drought in 2003 reported by *Ciais et al.* [2005]. The difference might be explained by the initial phase of establishment of the plant community and thus, reduced competition for water, and by comparatively high soil moisture during spring (Table 1). In 2005, the low rainfall sum, high soil temperatures coupled with low soil moisture obviously reduced productivity and thus, aboveground N storage (Table 1 and Figure 1). Despite this strong weather impact, the relationship between plant diversity and aboveground N storage or N availability in soil was comparable to the other years (Figure 2). Therefore, we infer that weather conditions play a role in determining the absolute values of aboveground N storage and N availability in soil, but not the strength of the plant diversity effect. However, much longer time series also including time lag considerations are needed to support our hypothesis.

[26] None of the studied N pools (aboveground N storage, NO_3-N and NH_4-N availability in soil) increased or decreased through time (Figure 1) which was in line with the observations of *Oelmann et al.* [2007b] for the concentrations of the various N species in mineral soil solution for the first 2 years of The Jena Experiment. Any trend in plant diversity effects can be attributed to counterbalancing effects of aboveground or belowground N fluxes or storage. We observed a consistent relationship between plant diversity and aboveground N storage which increased through time (Figure 2 and Table 2) in accordance with similar relationships for productivity at our site [*Marquard et al.*, 2009]. Contrastingly, *van Ruijven and Berendse* [2009] reported that the slope of the relationship between plant diversity and productivity saturated after 3 years. The difference between the results of *van Ruijven and Berendse* [2009] and those of our study might be related to the comparatively low number of species in the high-diversity treatment (eight-species mixtures) of *van Ruijven and Berendse* [2009] resulting in less required time until niche occupation both aboveground and belowground was reached. Similar to our results, in a meta analysis of several manipulated biodiversity experiments, effects of plant diversity on productivity increased through time [*Cardinale et al.*, 2007]. Aboveground N storage in diverse mixtures was closely linked to the biomass of legumes for our study site [*Oelmann et al.*, 2007b] and thus, maximum aboveground N storage will be determined by aboveground niche occupation (productivity) and additional sources of N supply of legumes (N_2 fixation, estimated to range 4.2 ± SD 1.8 g N m^{-2} yr^{-1} for the whole experimental area by *Oelmann et al.* [2007a]). As both productivity and N_2 fixation are expected to saturate through time (maximized under equilibrium conditions), we would predict a saturating slope of the relationship between plant diversity and aboveground N storage in the future.

[27] Mineral N (N_{min}) availability in soil among other factors is controlled by microbial activity and thus, by abiotic conditions (pH, which varies little in our experiment, soil moisture and soil temperature). In the first year after establishment of our experiment, plant diversity negatively correlated with NO_3-N availability in soil (Figure 2 and Table 3) in line with findings of other authors suggesting efficient resource use associated with depletion of nutrient

concentrations in soil [*Tilman et al.*, 1997; *Hooper and Vitousek*, 1998; *Scherer-Lorenzen et al.*, 2003; *Palmborg et al.*, 2005]. In the following 4 years (eight sampling campaigns), we never again observed a relationship between plant diversity and NO_3-N availability in soil. We cannot completely rule out the possibility that such a relationship might have occurred in deeper soil layers (>15 cm). However, during the first 2 years we additionally sampled the 15 to 30 cm layer and did not find any effect of plant diversity ($p > 0.05$, data not shown). Availability of NH_4-N in soil showed an opposite pattern characterized by (1) a positive correlation with plant diversity and (2) the occurrence of this relationship toward the end of our study period (Figure 2 and Table 4). Such a relationship has not yet been published, possibly mainly caused by the short duration of experiments focusing on nutrient availability in soil [*Tilman et al.*, 1997; *Hooper and Vitousek*, 1998; *Scherer-Lorenzen et al.*, 2003; *Palmborg et al.*, 2005].

[28] From a soil-focused point of view, the opposing temporal trends of the relationship between plant diversity and NO_3-N and NH_4-N availability in soil at first glance seemed to indicate a shift in highly diverse mixtures from increased resource use (depletion of NO_3-N in soil) during the first year to decreased resource use (accumulation of NH_4-N in soil) in the following years. However, consistently higher aboveground N storage of diverse mixtures indicated that highly diverse mixtures maintained increased aboveground N storage throughout the study period similarly to productivity [*Marquard et al.*, 2009]. Therefore, instead of direct effects by plant uptake, indirect effects such as processes in soil must be responsible for the observed pattern. Positive feedbacks of plant diversity on abiotic or biotic soil parameters can explain both the disappearance of a negative correlation and afterward, the establishment of a positive correlation with plant diversity. As an abiotic driver of nutrient release, soil moisture was shown to increase with plant diversity in the upper soil layer [*Caldeira et al.*, 2001; *Verheyen et al.*, 2008], thus possibly also balancing soil temperature amplitudes. Increased soil moisture and balanced soil temperatures would also increase net N release rates (sum of NH_4-N, NO_3-N, and dissolved organic N species) in diverse mixtures. Plant diversity was positively related to soil moisture in our experiment [*Kreutziger* 2006]. Higher soil moisture in highly diverse mixtures might, however, also result in reduced nitrification [*Hutchinson et al.*, 1993; *Feig et al.*, 2008], thereby increasing NH_4-N availability in soil. If this was the only mechanism, NO_3-N availability in soil would be negatively correlated with plant diversity throughout the study period, which was not the case. However, another important factor controlling N release in soil is total N storage which is in turn mainly controlled by litter input and decomposition rates [*Dybzinski et al.*, 2008; *Fornara and Tilman*, 2008; *Fornara et al.*, 2009]. Based on the accumulation of total N in soil between 2002 and 2006, we showed that N mineralized from total N accumulated in soil could compensate the initial depletion of NO_3-N availability in soil in highly diverse mixtures (Figures 1 and 3c). Third, the increasing establishment of a microbial community that is adapted to grassland sites [*Eisenhauer et al.*, 2010] and that efficiently transforms N species in soil might further promote increased net N release from soil through time.

[29] Because aboveground N storage showed no temporal pattern and because microbial communities probably have nearly reached equilibrium toward the end of the study period, the accumulation of total N storage in soil is the main control of the relationship between plant diversity and availability of mineral N species in soil. However, the source of the additional N, which is increasingly stored in soil in highly diverse mixtures is unknown. One possible source is gaseous deposition from the urban atmosphere of the study site which is rich in gaseous N compounds assimilated via the stomata (NH_3, NO_x, positive correlation with plant diversity driven by LAI) or N uptake from groundwater which might also be N-loaded because of the proximity of urban activities (positive correlation driven by spatial complementarity in highly diverse mixtures). The total deposition of 2.3 g N m^{-2} yr^{-1} measured at our study site by *Oelmann et al.* [2007a], however, was too small to explain the N gain in soil.

[30] We propose the following scenario for further development of soil and plant N pools in relation to plant diversity. The increased N storage in soil might be driven by translocation processes in soil associated with land-use change that will result in a depth profile of total N storage in soil typical for grasslands in the future [*Steinbeiss et al.*, 2008]. Because of scarce literature on this topic, we can only speculate that the accumulation of N in soil of our study site with high clay contents will saturate within decades or even centuries which was observed for sandy soils [*Knops and Tilman*, 2000; *McLauchlan*, 2006; *McLauchlan et al.*, 2006]. After accumulation of N in soil will have reached equilibrium, assuming mass constancy we would expect that the increased removal of N with the harvest can no longer be counterbalanced by increased N accumulation in soil in highly diverse mixtures. Even if gross mineralization is then still positively related to plant diversity, the increased removal of N with the harvest will lead to increasing depletion of N_{min} and total N storage in soil with increasing plant diversity in the long run. At this time, the initial relationship between plant diversity and NO_3-N availability in soil might reappear.

[31] Considering ecosystem services, plant diversity might contribute to guarantee quality and quantity of harvested biomass in grasslands through time. However, in grasslands a diversity-related reduction of the risk of N leaching to deeper soil layers or to the groundwater can only be expected during the first time after conversion from arable soils and possibly again after organic matter turnover rates have reached organic matter input rates. Therefore, sowing diverse species mixtures instead of monocultures or two-species mixtures when converting arable land to grassland reduces NO_3 concentrations in soil solution and thus in groundwater (i.e., drinking water resources) shortly after the conversion. Furthermore, sowing of diverse mixtures has the potential to maintain biomass production at an elevated level if the fertilizer amount is reduced in the course of agricultural extensification or conversion to organic farming, which are common trends in the temperate zone worldwide.

[32] **Acknowledgments.** We thank the many people who helped with the management of the experiment and the many student helpers

who assisted with the plant sample preparation for N analysis. Thanks also to all the helpers who assisted during the weeding campaigns. The Jena Experiment is funded by the Deutsche Forschungsgemeinschaft (DFG, FOR 456), with additional support from the Friedrich Schiller University of Jena and the Max Planck Society.

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