Web Ecology 7: 87–93.

Influence of legumes on N cycling in a heathland in northwest Spain

Alexandra Rodríguez, Jorge Durán and Antonio Gallardo

Rodríguez, A., Durán, J. and Gallardo, A. 2007. Influence of legumes on N cycling in a heathland in northwest Spain. – Web Ecology 7: 87–93.

Nitrogen availability frequently limits plant growth in natural ecosystems. N-fixers should have a substantial competitive advantage in N-limited systems, and as a byproduct of their activity they should increase the quantity and availability of N in the system as a whole. However, this effect has rarely been quantified in natural ecosystems. Heathlands in northwest Spain are frequently occupied by legume scrubs. We tested whether the presence of these legumes affected the N cycle in these communities. Specifically, we addressed the following questions: is nitrogen availability higher beneath legume canopies than beneath non-legume canopies? Is soil microbial biomass acting as a sink of extra N mineralized beneath legume canopies? Does the presence of legume scrubs change the soil pools of labile N and P? Is N plant uptake different under N-fixer scrubs than under non-N-fixer scrubs?

To answer these questions, we sampled soil beneath the canopy of randomly selected individuals of *Erica umbellata*, *Ulex gallii,* and *Genista tridentata* twice during the growing season. Soil samples were analyzed for organic matter, NH_4-N, NO_3-N, DON, PO_4-P, N mineralization and nitrification rates, and soil microbial biomass-N. In addition, we estimated N uptake by plants and the N concentration in green tissue to compare internal N cycles between legume and non-legume scrubs. Nitrification rates, DON (dissolved organic nitrogen), soil NO_3 concentration, and N uptake were significantly higher beneath legume canopies. However, soil microbial biomass-N and extractable-P were significantly lower under legumes. Our results showed that the presence of legume scrubs modify the size of N pools and the dominant form of available N for plants, increasing spatial heterogeneity in mixed stands.

Rodríguez, A. (arodper2@admon.upo.es), Durán, J. and Gallardo, A., Dept of Physical, Chemical and Natural Systems, Pablo de Olavide Univ., ES – 41013 Seville, Spain.

The supply of nitrogen often limits the growth of plants, influencing the composition of plant communities, and ecosystem productivity (Boring et al. 1988, Vitousek and Howarth 1991). A number of processes tend to reduce the biological availability of N in ecosystems, notably the strong link between organic N and recalcitrant C compounds in soils, and the mobility of N out of ecosystems by hydrologic and atmospheric pathways (especially leaching and denitrification). However, the capacity of biological nitrogen fixers to convert N_2 to organic N is substantial where symbiotic N fixers are abundant, and enough to maintain N pools in ecosystems and to replenish N losses (Vitousek et al. 2002). Consequently, nitrogen fixation by autotrophs can be an important mechanism of nitrogen accumulation in ecosystems (Sprent and Sprent 1990). Plant species with symbiotic N_2-fixing bacteria can fix 50 to 200 kg N ha^{-1} y^{-1} in pure stands (Boring et al. 1988, Binkley et al. 1994, Cleveland et al. 1999). These fixation

Accepted 3 October 2007

ISSN 1399-1183

inputs can be greater than atmospheric N loading in industrial regions (Wright and Rasmussen 1998), and result in high leaching losses from N-saturated terrestrial ecosystems in polluted regions (Van Miegroet et al. 1992, Gundersen and Bashkin 1994).

N fixers can increase soil N content and cycling rates in pure stands or in mixtures with other species (Binkley et al. 1992, 1994, Rothe et al. 2002). High rates of nitrification and organic matter cycling under N-fixing species generally accelerate cation leaching and soil acidification when compared to non-fixers (Van Miegroet and Cole 1984, 1985, Bormann et al. 1994). Nitrogen-fixing plants may also affect other soil properties, including soil pH (Binkley and Sollins 1990), carbon accumulation (Cole et al. 1995), soil P fractions, and P cycling (Giardina et al. 1995, Compton and Cole 1998, Binkley et al. 2000). Lower available soil P concentrations may result under nitrogen-fixing species than under non-fixing species, because nitrogen-fixing species have high P requirements (Gressel et al. 1996). However, a consistent pattern of nitrogen-fixing species increasing or decreasing available soil P has not been established. The presence of some N_2-fixing species appears to increase available P in mixed stands (Giardina et al. 1995, Zou et al. 1995), but they decrease available P in pure stands (Cole et al. 1991, Compton et al. 1997).

Rastteter et al. (2001) showed that N fixation is favored when (1) the canopy is open, (2) available N levels in the soil are low, and (3) the soil is already well exploited by fine roots. Low biomass and thin soils of heathlands in northwest Spain should provide conditions that favor N fixation, and consequently N cycling should be substantially affected by the presence of legume scrubs. These heathlands are dominated by *Erica* species (*E. umbellata*, *E. cinerea*, *E. australis*), with the occasional presence of N-fixing legumes of the genus *Ulex* (*U. europaeus*, *U. minor* Roth, *U. gallii*) and *Genista* (*G. tridentata*). The presence of these legumes in heathlands is favored by disturbance, when conditions are optimal for the establishment of N-fixers (Vitousek and Field 1999, Casals et al. 2005). The presence of legumes may increase the leaching of N out of ecosystems, as this area is under increasing atmospheric N deposition (Klein et al. 2005). However, as suggested by McKey (1994), legumes have an N-demanding lifestyle requiring higher concentrations of N than plants in other families. This greater requirement for N do not depend on the way individual plants are acquiring N. Thus, when N atmospheric fixation is not possible, legumes must increase the plant N uptake from soil. Therefore, plant N uptake may be as high under N-fixers as under non-fixers and N leaching may not be enhanced by the presence of N-fixing plants.

This work is a first attempt to quantify the role of individual plants as a source of spatial heterogeneity in the N cycle. The increase in this spatial heterogeneity may have important implications for plant dynamics, as it may favor species with high nitrogen requirements, affecting ecosystem biodiversity and productivity. Specifically, we addressed the following questions: (1) is nitrogen availability higher beneath legume canopies than beneath non-legume canopies? (2) Is soil microbial biomass acting as a sink of the extra N mineralized beneath legume canopies? (3) Does the presence of legume scrubs change the soil pools of labile N and P (NH_4-N, NO_3-N, PO_4-P, and DON)? (4) Is N plant uptake different under N-fixer scrubs than under non-N-fixing scrubs?

Material and methods

Study site

The study area was located on the Galiñeiro mountain (Galicia, northwest Spain, 42°08′12.6′′N, 8°41′59′′W) at 700 m altitude. The climate is warm-temperate with a slight Mediterranean influence, with little precipitation during the summer months. Mean annual precipitation is about 2000 mm and the mean annual temperature is about 12.2°C. Soils of the study area are classified as Lithic Leptosol (FAO 1988). These shallow soils are slightly acidic (pH 4.5), and are derived from the weathering of biotite gneiss bedrock. Beneath a thin litter layer lies a 10-cm-deep organic horizon with a 4.5% soil organic carbon content. Total plant cover (80%) was dominated by *Erica umbellata*, *Ulex gallii*, and *Genista tridentata* that accounted for 60%, 30%, and 10%, respectively, of the total plant cover. The area has been exposed to recurrent fires during the last century, although there were no recorded fires in the study site over the last 20 years. This area was also exposed to moderate-to-low herbivory rate, mainly by horses and goats.

Field methods

A 2-ha plot (slope 3–5%) was located in the study area in July 2003. Ten individuals of each of the three dominant scrub species were randomly chosen within this plot. The top 10 cm of the soil profile beneath the canopy of the selected individuals was sampled twice in the growing season with a 20-day interval (9 July and 29 July). Net nitrification and N-mineralization rates were measured using the procedure described by Eno (1960). For each soil sample, the top 10 cm of soil A horizon was removed and placed in a plastic bag, then reburied in the forest floor for a 20-day incubation. Net N mineralization was defined as the net increase in NH_4-N + NO_3-N over the incubation interval; of this, the net increase in NO_3-N was used to indicate net nitrification rate. Samples were collected at the beginning and end of the incubation to establish concentrations of NO_3-N and NH_4-N in unincubated samples. Mean gravimetric soil water content and mean temperature during the incubation was 18% and 20°C, respectively. A metallic cylinder of 5 cm diameter × 15 cm high

was used for sampling. Samples of above- and belowground plant tissues were collected from each selected individual on the second sampling date.

Lab analysis

Each soil sample was sieved (<2 mm) in field-moist condition, and then analyzed for organic matter, mineral N (ammonium and nitrate), microbial biomass-N, and DON. In the samples, the organic matter was estimated by loss-on-ignition (Nelson and Sommers 1996). In order to extract mineral nitrogen from soil, 10 g of sample was shaken with 100 ml of a 2 M KCl solution for 1 h, and the suspension was filtered through a 0.45 μm Millipore filter. Aliquots of this solution were transferred in three batches on to microplates. Nitrate was reduced to ammonium by allowing the Devarda alloy to react overnight with the extract. NO_3^- reduction efficiency by Devarda alloy was close to 100%, and corrections with blanks, NO_3^-, and NH_4^+ standards were carried out in each run. Samples were then transferred to another microplate and mineral nitrogen content in these samples was determined by colorimetry, using a microplate reader (Sims et al. 1995). Soil phosphate was extracted at a ratio of 20:1 with 2.5% v/v acetic acid and measured by colorimetry (Allen et al. 1986). Soil microbial biomass-N was analyzed in incubated and unincubated soil samples using the fumigation-extraction method as outlined by Brookes et al. (1985). Fresh soil subsamples were exposed to chloroform for 5 days. Five g of soil were extracted with 100 ml of 0.5 M K_2SO_4, and filtered through 0.45 μm Millipore filters. Subsamples extracted with 0.5 M K_2SO_4 immediately before fumigation served as controls. Total N from non-fumigated samples was subtracted from fumigated ones in order to estimate microbial biomass-N. Total N in the extracts was calculated using a persulfate oxidation technique, which was originally developed for the determination of total N in seawater (D'Elia et al. 1977). This method has proven to be a rapid and efficient way to measure total nitrogen in K_2SO_4 extracts (Cabrera and Beare 1993, Hossain et al. 1993). Nitrate in the digest was reduced to ammonium and analyzed by colorimetry as described above. DON was analyzed by persulfate oxidation of non-fumigated K_2SO_4 extracts and substraction of mineral N from those extracts (Cabrera and Beare 1993, Doyle et al. 2004).

All results were expressed as milligrams per kilogram of soil or milligrams per kilogram of soil per day (N net mineralization, net nitrification, and plant N-uptake), based on oven-dried soil.

Plant tissue samples (leaves, stems, and roots, < 2 mm diameter) were air-dried, milled without heating, and stored for subsequent analyses. For plant N analysis, samples were digested in selenous sulfuric acid (Walinga et al. 1995). Diluted aliquots of the digestion were analyzed for N by colorimetry (indophenol blue method) using a microplate reader (Sims et al. 1995), and for P by colorimetry (molybdenum blue method, Allen et al. 1986).

Numerical analysis

Nitrogen uptake for each selected individual was estimated by mass-balance method. The total N uptake over the 20-day period was estimated as follows:

$$Np = \Delta \text{ mineral-N} - Nm - \text{denitrification} - \text{N leaching}$$

where Np was plant N uptake during the 20-day period, Δ mineral-N was the increase of NH_4-N plus NO_3-N observed in the incubated samples (without root uptake) compared to the unincubated samples (with root uptake). Nm was microbial N uptake during the 20 days, calculated as the increase of microbial biomass-N in the incubated samples compared to the unincubated samples.

On account of the lack of precipitation in the 20-day incubation period and the high redox potential of soils, denitrification and N leaching were taken as zero.

We compared the differences in plant N and P concentration as well as soil variables between *Erica umbellata* versus the two legume species (*U. gallii* and *G. tridentata*). Differences among the three species were also tested by using permutation test, but we combined results for the two legume species because differences between them were not significant. The Shapiro–Wilk normality test on the plant and soil variables showed that, in most cases, data were not significantly adjusted to a normal or log-normal distribution. Consequently, data were ranked and analyzed using the non-parametric Kruskal–Wallis statistical test. Statistical analyses were performed with the statistical software R (R Development Core Team 2006).

Results

Soil organic matter concentrations beneath N-fixers were not significantly different from values found under non-fixing scrub canopies (Table 1). Differences in the soil inorganic nutrient pools between N-fixers and non-fixers yielded mixed results. No differences were found in soil NH_4-N concentrations, but NO_3-N was significantly higher and soil PO_4-P was significantly lower beneath N-fixing species than under non-N-fixers (Table 1). Mean soil NO_3-N concentration was between 2 and 4 times higher under N-fixers, while mean soil PO_4-P decreased between 50% and 100% under N-fixers. The microbial biomass-N was significantly 35% lower under N-fixers than under non-fixers. Differences in DON concentration were not consistent on the two sampling dates; no differences were found for DON on the first sampling date, but higher levels of DON under N-fixers were found on the second sampling date (Table 1). Soil DON concentration was of the same order of magnitude as soil NH_4-N and NO_3-N.

The difference in the net N mineralization rate between N-fixers and non-fixers was not significant (Table 2); however, differences were found in the net nitrification rate

Table 1. Mean soil variables measured under the canopy of N-fixer (n = 20) and non-fixer (n = 10) species on two sampling dates (top 10 cm of soil profile). Values between brackets are standard deviations. MB-N = microbial biomass-N. DON = dissolved organic nitrogen.

		9 July			29 July		
	Units	N-fixers	Non-fixers	p-value	N-fixers	Non-fixers	p-value
Organic matter	(%)	9.85 (2.97)	10.40 (3.40)	0.6696	9.59 (2.90)	9.44 (2.01)	0.8843
NH_4-N	mg kg^{-1}	11.09 (5.81)	12.16 (12.79)	0.3353	13.0 (9.34)	10.67 (12.64)	0.5712
NO_3-N	mg kg^{-1}	22.57 (7.45)	6.56 (4.78)	0.0000	13.28 (5.37)	6.11 (6.17)	0.0049
PO_4-P	mg kg^{-1}	0.50 (0.30)	0.76 (0.33)	0.0414	0.42 (0.28)	0.93 (0.34)	0.0015
DON	mg kg^{-1}	21.34 (13.59)	27.91 (13.11)	0.2036	17.96 (7.67)	7.37 (6.81)	0.0018
MB-N	mg kg^{-1}	109.27 (59.29)	174.84 (78.34)	0.0209	42.98 (36.26)	66.23 (38.51)	0.0329

and the estimated plant N uptake rate (Table 2). The net nitrification rate was an order of magnitude higher under N-fixers than under non-fixers, and N uptake by N-fixing plants was three times higher than that of non-N-fixing plants. Plant N uptake was of similar magnitude to the net mineralization rate in *Erica umbellata*, but higher than the net mineralization rate under the N-fixing species ($p< 0.05$).

Above- and belowground plant N concentrations in N-fixers were significantly higher than in non-fixers tissues (Table 3). However, differences in above- and belowground plant P concentration were not significant (Table 3).

Discussion

The main effect of the presence of legume species was the increased nitrification rate, NO_3 concentration and N uptake rate under their canopies. Contrary to our expectations, the microbial biomass did not act as an extra sink for N under legumes, and soil organic matter and overall N and P availability were not increased under N-fixer species. Therefore, our results highlighted that changes in microbial activity (less ammonification, more nitrification), and changes in microbial populations (as suggested by less N in microbial biomass) were more important that changes in total soil organic and inorganic pools. However, mean distance between plants were about 1 m, and we can not discard that legumes and *Erica* plants were mutually influenced by each other, decreasing the expected differences between these two types of plants.

Comparisons by some authors of N-fixers versus non-N-fixers have found 20%–100% more soil organic C under N-fixers (Johnson 1992, Cole et al. 1995, Rhoades et al. 1998, Kaye et al. 2000, Resh et al. 2002), suggesting that communities with pure and mixed species that include N-fixers appear to be an option for soil C sequestration purposes. However, in agreement with our results, other authors found little effect of N-fixing species on organic matter of surface soil (Stock et al. 1995, Haubensak and Parker 2004). In our study area, the effects of N-fixers on soil organic matter may be obscured by losses of organic matter from soils due to the effect of recurrent fires (Roscoe et al. 2000).

Haubensak and Parker (2004) found that net mineralization and nitrification rates were significantly higher in *Cytisus*-invaded soils compared to non-invaded soils in prairies of western Washington. We find differences only in the net nitrification rate, indicating that only nitrifica-

Table 2. Means and standard deviations of soil N mineralization, nitrification, and plant N uptake rates and the soil C-to-N ratio estimated under the canopy of N-fixer (n = 20) and non-fixer (n = 10) scrubs in a heathland in northwest Spain (top 10 cm of soil profile).

	Units	N-fixers	Non-fixers	p-value
Net N-mineralization rate	mg N kg^{-1} soil day^{-1}	0.88 (0.98)	0.43 (0.83)	0.2233
Net nitrification rate	mg N kg^{-1} soil day^{-1}	0.32 (0.39)	0.016 (0.22)	0.0108
Plant N-uptake rate	mg N kg^{-1} soil day^{-1}	1.25 (0.94)	0.41 (0.39)	0.0121
C-to-N ratio	——	11.74 (2.75)	13.74 (2.14)	0.0015

Table 3. Means and standard deviations of N and P concentrations of above- and belowground tissues of N-fixer (n = 20) and non-fixer (n = 10) scrubs in a heathland in northwest Spain.

	Units	N-fixers	Non-fixers	p-value
Aboveground plant N	mg g^{-1}	14.39 (1.56)	9.75(1.73)	< 0.0000
Belowground plant N	mg g^{-1}	11.47 (1.58)	7.69 (1.94)	0.0002
Aboveground plant P	mg g^{-1}	0.60 (0.12)	0.55 (0.16)	0.2710
Belowground plant P	mg g^{-1}	0.26 (0.17)	0.21 (0.11)	0.5522

tion was favored by the presence of legumes. Competition for NH_4 between nitrifiers and other soil microbes has been postulated as the cause for inhibition of nitrification rates observed in several ecosystems (Binkley and Vitousek 1991). In our study site, the different quality of litter and organic matter accumulated under N-fixers may have favored the nitrifier activity. This assumption implies that under a high C-to-N ratio, sufficient C-substrate exists to enable heterotrophic microbial growth (NH_4 immobilization) to dominate over autotrophic growth (NH_4^+ oxidation). But when the C-to-N ratio is low (under N-fixers), then heterotrophs can no longer immobilize NH_4^+ but nitrifiers can still oxidize NH_4^+ to NO_3^- and consequently it accumulates. Differences in moisture levels or pH between soils cannot explain the differences in nitrification rates, since these variables of the two soils were not significantly different (data not shown). The enhancement of the nitrification rate and soil NO_3 concentration, but not the mineralization rate or soil NH_4 under legumes, agrees with the conclusion of Haubensak and Parker (2004) that the impact of legume invasion may be influenced or tempered by chemical or microbial effects on the soil rather than simply increasing labile N. This soil N enhancement effect has been demonstrated in a number of other studies of N-fixers (Vitousek and Walker 1989, Stock et al. 1995, Maron and Connors 1996). Enhancement of soil N quality by N-fixers may appear to be highly predictable, yet this effect can vary strongly even between closely related species (Stock et al. 1995). The highest nitrification rate and NO_3-N concentration found under N-fixers in this study may lead to higher losses of N through leaching from the ecosystem, as found in systems of high nitrogen fixation (Binkley et al. 1992, Compton et al. 2003).

Extractable phosphate was also significantly lower under N-fixing species, suggesting that N-fixers depleted soil phosphate due to a higher demand for soil P. There are two reasons why symbiotic N fixers might have a greater requirement for P than non-fixers. First, building and/or maintaining the symbiosis, and/or the fixation process itself, requires more P than is needed by non-fixing organisms. Second, fixers could have a P-demanding lifestyle to support the N-demanding lifestyle that McKey (1994) proposes to be driving the legume-rhizobium symbiosis. A commitment to high N levels in organisms may entrain a commitment to high P levels as well (Vitousek et al. 2002). The lack of significant differences in P concentrations in plant tissues found in our plant community did not support this last alternative.

The results presented here confirm the previous studies showing that DON represents a major soluble N pool in soil and reaffirms the need to include DON in ecosystem N budgets and N cycling studies (Murphy et al. 2000, Siemens and Kaupenjohann 2002, Jones et al. 2004). Differences in DON between fixers and non-fixers were significant only at the second sampling date, with higher levels beneath the N-fixer canopy. The loss of DON is an important route for N loss in terrestrial ecosystems and can account for a significant fraction of N losses to streams, despite the ecosystem demand for N (Perakis and Hedin 2002, Vitousek et al. 2002, Neff et al. 2003). Both soil NO_3 and DON diffuse better in soil than soil NH_4, facilitating N uptake for plants under legumes and nearby plants, but also increasing the risk of N losses from the ecosystem.

The lower levels of microbial biomass-N under legumes may indicate changes in the C-to-N ratio of the microbial population more than a decrease in total microbial biomass under legumes. Indeed, the higher nitrification rate under legumes is an indicator that the microbial population under N-fixers may be different from those under non-fixers. Less N immobilized by soil microbes may enhance N losses from the ecosystem, because rapid microbial NO_3^-, NH_4^+, and DON assimilation may be an important mechanism for N retention. These forms of nitrogen assimilated by microbial biomass can be released as organic N that is less susceptible to leaching and denitrification (Yu et al. 2003). The lower microbial biomass-N and higher nitrification rate found under legumes is consistent with the observation made by Binkley et al. (1992) that N-fixers typically accelerate N turnover more than they increase N capital.

Our results supported McKey's (1994) suggestion that legumes in general have an N-demanding lifestyle – that they require higher concentrations of N than do plants in other families. He suggests that this greater requirement for N should be observed, whether or not an individual plant is acquiring its N by fixation, and whether or not an individual species of legume also has the capacity to fix N. Our mass balance approach indicated that N uptake by roots was higher under the two N-fixing species than under *E. umbellata*. Besides, although we do not have experimental support, indirect evidence suggested that atmos-

pheric N fixing is operating in our plant community. First, nodules were apparent in roots of these legumes, indicating their ability to fix atmospheric N on a short-term basis. Second, Rastetter et al. (2001) showed that legume N-fixation is more economical than soil N uptake when the canopy is open, the soil is well exploited by roots, soil inorganic N concentrations are low, and other soil resources (P, water) are readily available. Most of these conditions are fulfilled in the studied heathland community, suggesting that N-fixing may be operating in our plant community to some extent.

Our results showed that the presence of legumes scrubs modify the size of N pools and the dominant form of available N for plants, increasing spatial heterogeneity in mixed stands, which may allow coexistence of plant species with different N requirements.

Acknowledgements – This paper was financed by the Spanish Ministry of Science and Education (REN2003-08620-C02-01) and the Galician (NW Spain) government which provided a fellowship to Alexandra Rodríguez.

References

Allen, S. E. et al. 1986. Chemical analysis. – In: More, P. D. and Chapman, S. B. (eds), Methods in plant ecology. Blackwell, pp. 285–345.

Binkley, D. and Sollins, P. 1990. Factors determining differences in soil pH in adjacent conifer and alder–conifer stands. – Soil Sci. Soc. Am. J. 54: 1427–1433.

Binkley, D. and Vitousek, P. M. 1991. Soil nutrient availability. – In: Pearcy, R. W. et al. (eds), Plant physiological ecology. Field methods and instrumentation. Chapman and Hall, pp. 75–96.

Binkley, D. et al. 1992. Biogeochemistry of adjacent conifer and alder–conifer stands. – Ecology 73: 2022–2033.

Binkley, D. et al. 1994. Nitrogen fixation by red alder: biology, rates, and controls. – In: Hibs, D. E. et al. (eds), The biology and management of red alder. Oregon State Univ. Press, pp. 57–72.

Binkley, D. et al. 2000. Soil phosphorus pools and supply under the influence of *Eucalyptus saligna* and nitrogen-fixing *Albizia facaltaria*. – For. Ecol. Manage. 128: 241–247.

Boring, L. R. et al. 1988. Sources, fates and impacts of nitrogen inputs to terrestrial ecosystems: review and synthesis. – Biogeochemistry 6: 119–159.

Bormann, B. T. et al. 1994. The influences of red alder on soils and long-term ecosystem productivity. – In: Hibbs, D. E. et al. (eds), The biology and management of red alder. Oregon State Univ. Press, pp. 47–56.

Brookes, P. C. et al. 1985. Chloroform fumigation and the release of soil nitrogen; a rapid direct extraction method to measure microbial biomass nitrogen in soil. – Soil Biol. Biochem. 17: 837–842.

Cabrera, M. L., and Beare, M. H. 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. – Soil Sci. Soc. Am. J. 57: 1007–1012.

Casals, P. et al. 2005. Short-term nitrogen fixation by legume seedlings and resprouts after fire in Mediterranean old-fields. – Biogeochemistry 76: 477–501.

Cleveland, C. C. et al. 1999. Global patterns of terrestrial biological nitrogen (N_2) fixation in natural ecosystems. – Global Biogeochem. Cycles 13: 623–645.

Cole, D. W. et al. 1991. Changes in soil properties and site productivity caused by alder. – Water Air Soil Pollut. 54: 231–246.

Cole, D. W. et al. 1995. Comparison of carbon accumulation in Douglas fir and red alder forests. – In: McFee, W. W. and Kelly, J. M. (eds), Carbon forms and functions in forest soils. Soil Sci. Soc. Am., pp. 527–546.

Compton, J. E. and Cole, D.W. 1998. Phosphorus cycling and soil P fractions in Douglas-fir and red alder stands. – For. Ecol. Manage. 110: 101–112.

Compton, J. E. et al. 1997. Leaf element concentrations and soil properties in successive rotations of red alder (*Alnus rubra*). – Can. J. For. Res. 27: 662–666.

Compton, J. E. et al. 2003. Nitrogen export from forested watersheds in the Oregon coast range: the role of N_2-fixing red alder. – Ecosystems 6: 773–785.

D'elia, C. F. et al. 1977. Determination of total nitrogen in aqueous samples using persulfate digestion. – Limnol. Oceanogr. 22: 760–764.

Doyle, A. et al. 2004. Persulfate digestion and simultaneous colorimetric analysis of carbon and nitrogen in soil extracts. – Soil Sci. Soc. Am. J. 68: 669–676.

Eno, C. F. 1960. Nitrate production in the field by incubating the soil in polyethylene bags. – Soil. Sci. Soc. Am. Proc. 24: 277–279.

FAO 1988. FAO/Unesco soil map of the world, revised legend, with corrections and updates. World Soil Resour. Rep. 60. – FAO, Rome.

Giardina, C. P. et al. 1995. Alders increase soil-phosphorus availability in a Douglas-fir plantation. – Can. J. For. Res. 25: 1652–1657.

Gressel, N. et al. 1996. Linkages between phosphorus transformation and carbon decomposition in a forest soil. – Biogeochemistry 33: 97–123.

Gundersen, P. and Bashkin, V. N. 1994. Nitrogen cycling. – In: Moldan, B. and Cerny, J. (eds), Biogeochemistry of small catchments: a tool for environmental research. Wiley, pp. 255–2 83.

Haubensak, K. A. and Parker I. M. 2004. Soil changes accompanying invasion of the exotic shrub *Cytisus scoparius* in glacial outwash prairies of western Washington. – Plant Ecol. 175: 71–79.

Hossain, A. et al. 1993. Acid-peroxide digestion procedure for determining total nitrogen in chloroform-fumigated and non-fumigated soil extracts. – Soil Biol. Biochem. 25: 967–969.

Jones, D. L. et al. 2004. Role of dissolved organic nitrogen (DON) in soil N cycling in grassland soils. – Soil Biol. Biochem. 36: 749–756.

Johnson, D. W. 1992. Effects of forest management on soil carbon storage. – Water Air Soil Pollut. 64: 83–120.

Kaye, J. P. et al. 2000. Nutrient and carbon dynamics in a replacement series of *Eucalyptus* and *Albizia* trees. – Ecology 81: 3267–73.

Klein, H. et al. 2005. Transboundary air pollution by main pollutants (S, N, O_3) and PM. Spain. EMEP status rep. 2005. – Norw. Meteorol. Inst.

Maron, J. L. and Connors, P. G. 1996. A native nitrogen-fixing shrub facilitates weed invasion. – Oecologia 105: 302–312.

McKey, D. 1994. Legumes and nitrogen: the evolutionary ecology of a nitrogen-demanding lifestyle. – In: Sprent, J. I. and McKey, D. (eds), Advances in legume systematics 5: the nitrogen factor. R. Bot. Gard., pp. 211–228.
Murphy, D. V. et al. 2000. Soluble organic nitrogen in agricultural soils. – Biol. Fertility Soils 30: 374–387.
Neff, J. C. et al. 2003. Breaks in the cycle: dissolved organic nitrogen in terrestrial ecosystems. – Front Ecol. Environ. 1: 205–211.
Nelson, D. W. and Sommers, L. E. 1996. Total carbon, organic carbon and organic matter. – In: Methods of soil analysis. Part 3, Chemical methods. Soil Sci. Soc. Am. and Am. Soc. Agron. SSSA Book Ser. no. 5.
Perakis, S. S. and Hedin, L. O. 2002. Nitrogen loss from unpolluted south American forests mainly via dissolved organic compounds. – Nature 415: 416–419.
R development core team. 2006. R: A language and environment for statistical computing. – R Found. for Stat. Computing.
Rastetter, E. B. et al. 2001. Resource optimization and symbiotic nitrogen fixation. – Ecosystems 4: 369–388.
Resh, S. C. et al. 2002. Greater soil carbon sequestration under nitrogen-fixing trees compared with *Eucalyptus* species. – Ecosystems 5: 217–231.
Rhoades, C. C. et al. 1998. Effect of pasture trees on soil nitrogen and organic matter: implications for tropical montane forest restoration. – Restor. Ecol. 6: 262–70.
Roscoe, R. et al. 2000. Effects of fire on soil organic matter in a "cerrado sensu stricto" from southeast Brazil as revealed by changed in delta13C. – Geoderma 95: 141–160.
Rothe, A. et al. 2002. Soil carbon and nitrogen changes under Douglas-fir with and without red alder. – Soil Sci. Soc. Am. J. 66: 1988–1995.
Siemens, J. and Kaupenjohann, M. 2002. Contribution of dissolved organic nitrogen to N leaching from four German agricultural soils. – J. Plant Nutr. Soil Sci. 165: 675–681.
Sims, G. K. et al. 1995. Microscale determination of inorganic nitrogen in water and soil extracts. – Commun. Soil Sci. Plant Anal. 26: 303–316.
Sprent, J. I. and Sprent, P. 1990. Nitrogen-fixing organisms. – Chapman and Hall.
Stock, W. D. et al. 1995. Impacts of invading N_2-fixing *Acacia* species on patterns of nutrient cycling in two Cape ecosystems: evidence from soil incubation studies and 15N natural abundance values. – Oecologia 101: 375–382.
Van Miegroet, H. and Cole, D. W. 1984. Impact of nitrification on soil acidification and cation leaching in a red alder ecosystem. – J. Environ. Qual. 13: 586–590.
Van Miegroet, H. and Cole, D. W. 1985. Acidification sources in red alder and Douglas fir soils – importance of nitrification. – Soil Sci. Soc. Am. J. 49: 1274–1279.
Van Miegroet, H. et al. 1992. Nitrogen distribution and cycling. – In: Johnson, D. W. and Lindber, S. E. (eds), Atmospheric deposition and forest nutrient cycling: a synthesis of the integrated forest study. Springer, pp. 178–196.
Vitousek, P. M. and Field C. B. 1999. Ecosystem constraints to symbiotic nitrogen fixers: a simple model and its implications. – Biogeochemistry 46: 179–202.
Vitousek, P. M. and Howarth, R. W. 1991. Nitrogen limitation on land and in the sea: how can it occur? – Biogeochemistry 13: 87–115.
Vitousek, P. M. and Walker, L. R. 1989. Biological invasion by *Myrica faya* in Hawaii: plant demography, nitrogen fixation, ecosystem effects. – Ecol. Monogr. 59: 247–265.
Vitousek, P. M. et al. 2002. Towards an ecological understanding of biological nitrogen fixation. – Biogeochemistry 57/58: 1–45.
Walinga, L. et al. 1995. Plant analysis manual. – Kluewer.
Wright, R. F. and Rasmussen, L. 1998. Introduction to the NITREX and EXMAN projects. – For. Ecol. Manag. 101: 1–7.
Yu, Z. et al. 2003. Mineral and dissolved organic nitrogen dynamics along a soil acidity-fertility gradient. – Soil Sci. Soc. Am. J. 67: 878–888.
Zou, X. et al. 1995. Effects of dinitrogen-fixing trees on phosphorus biogeochemical cycling in contrasting forests. – Soil Sci. Soc. Am. J. 59: 1452–1458.