Nitrogen Cycling in a Mature Mountainous Beech Forest

Panagiotis Michopoulos, George Baloutsos and Anastassios Economou

The nitrogen cycle in a mature, mountainous European beech (Fagus sylvatica) forest in Greece was examined for two hydrological years, 2001–2002 and 2002–2003. Bulk deposition was 1383 mm in 2001–2002 and 2392 mm in 2002–2003. Winter temperatures were mild in the first year and low in the second year. Despite these climatological differences, the inorganic N inputs to the forest floor, by means of throughfall and stemflow, were quite similar for the two years, i.e. 12.4 and 14.6 kg ha\(^{-1}\) yr\(^{-1}\). Litterfall production was significantly (p < 0.05) higher in the second year but the N amounts in litterfall did not differ. The ratio of N/P in foliar tissue did not change significantly in 2003 compared with ratio values in the last years. The N amounts used for the annual stem and branch increments are rather high preventing, in this way, some nitrogen from being recycled in the near future. The total soil N content to a depth of 80 cm amounted to more than 5000 kg ha\(^{-1}\), and the C/N ratio in the Oh horizon was approximately 15 but the beech forest did not appear susceptible to N leaching. The concentrations of ammonium and nitrate N in stream water did not reach high values reported in the literature, and did not differ significantly in the two hydrological years. The fluxes of inorganic N in throughfall plus stemflow were higher than those in stream water indicating N retention in soil. Another reason for N retention in the ecosystem is probably the large difference between N requirements and uptake indicating N deficiency. Despite the maturity of the beech trees, the low C/N ratio in the Oh horizon and the relatively high N content in soil, the forest can be considered to be neither saturated nor having reached a N saturation transition stage.

Keywords bulk deposition, Fagus sylvatica, litterfall, stemflow, stream water, throughfall
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Received 22 January 2007 Revised 31 July 2007 Accepted 5 October 2007
Available at http://www.metla.fi/silvafennica/full/sf42/sf421005.pdf
1 Introduction

It is well established that nitrogen (N) is the macronutrient often limiting the growth of trees on forest soils (Vitousek and Farrington 1997). This happens not because of N scarcity in forest soils but because of its limited availability. Due to its importance, considerable information exists on N cycling in forest ecosystems (Carlyle 1986, Keeney 1980, Jussy et al. 2004). Cole and Rapp (1981) compiled lists of N amounts stored in various compartments of forest ecosystems all over the world. Forest tree species have adapted to low soil N status by developing mechanisms for translocating N from old leaves into the tree during leaf senescence, storage over winter, and then translocating into new leaves the following year. The so-called “nitrogen use efficiency” refers to this particular ability (Vitousek 1982).

Nitrogen deposition has remained unchanged or even increased in European forests due to emissions of NOx from industrial and automotive combustion and NH3 from agricultural activities. The effects of elevated N deposition on forest ecosystems were first studied in northern temperate forests (Aber et al. 1998, Miegroet et al. 1992) and gave rise to the concept of N saturation. The impact of chronic N deposition in forests can have far reaching consequences. It can promote deficiencies in other nutrients, such as P and K (Carreira et al. 1997). High levels of N deposition can promote increased mineralization of soil organic matter, resulting in enhanced N uptake (Vestgarden 2001) and increased turnover rates of organic matter. Boweden et al. (2004) found that chronic N additions can reduce soil microbial activity and thus forest productivity. The term “nitrogen saturation” refers to the exceeded N assimilation capacity of forested ecosystems and enhanced nitrification is a symbol of saturation. High concentrations of nitrates in soil solution result in cation leaching, primarily Ca and Al (Fernandez et al. 1999).

In order to assess the risk of N leaching, researchers have tried to set thresholds of ecological parameters based on data from a large number of forested plots (Table 1).

Mainly, two predictors were found to significantly correlate with nitrate output. The first is the N in throughfall. Below a deposition value of about 10 kg of inorganic N ha⁻¹ yr⁻¹, no significant leaching occurs, at intermediate levels of 10–25 kg ha⁻¹ yr⁻¹ leaching occurs at some sites and above 25 kg ha⁻¹ yr⁻¹, significant leaching occurs at all sites (Dise and Wright 1995). The second predictor is the ratio of C/N in the soil organic horizon. Above a ratio value of 25, no significant nitrate leaching occurs (Macdonald et al. 2002). Also N pool size and change in foliar N concentration have been suggested as criteria (Table 1).

Mature forest ecosystems are more prone to N saturation than aggrading forests, as biomass accumulation is low. In these types of forests, N inputs should equal outputs unless atmospheric deposition supplies forest with high amounts of N (Vitousek and Reiners 1975).

The objectives of this work were: first to assess N cycling in two successive hydrological years, 2001–2002, 2002–2003 and second to test the hypothesis that N leaching is significant in a mature mountainous beech forest, having a C/N ratio in soil less than 25 and receiving intermediate levels of N deposition.

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<tr>
<td>C/N &lt; 25 in organic soil horizons</td>
<td>N Pool in soil &gt;5000 kg ha⁻¹ for a soil with C/N ratio &lt; 20 in organic soil horizons</td>
<td>Throughfall flux&gt;10 kg ha⁻¹ yr⁻¹</td>
<td>Change of foliar N concentration over time</td>
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</table>
2 Materials and Methods

2.1 Site Description

The beech forest under consideration grows in a mountainous watershed, 260 ha in size, located in central eastern Greece (Fig. 1) at an elevation range of 740–1420 m. The watershed is underlain by schist. The climate is inland Mediterranean with mean annual precipitation of 1626 mm and temperature of 11.1°C. These means were derived from 26 years of observation (1978–2003). The monthly precipitation amounts in the two hydrological years are presented in Fig. 2. Air and soil (at a depth of five cm) temperature was monitored by recording thermometers, approximately 30 m from the plot center. From both thermometers, monthly mean values were calculated from daily mean temperatures. In the first hydrological year (2001–2002), sub-zero air temperatures occurred in December and in the soil in January. For the second hydrological year (2002–2003), sub-zero temperatures occurred in February and March and in the soil in December to April (Fig. 3).

Due to these air temperatures, snow melting, in the first hydrological year started in February and in April in the second year. The dates of melting were certified by field observation.

The experimental plot has an area of 0.27 ha, an average slope of 33% and a northeastern aspect. The *Fagus sylvatica* (ssp. *Fagus moesiaca*) stand is even-aged (110–130 years old), with an average tree height of 27 m and canopy closure of 0.8 to 1.0. The ground vegetation is very sparse, occupying only 5% of the ground area and consisting of the herb species: *Galium retundifolium*, *Doronium orientale* and *Cyclamen graecum*. The soil at the experimental plot is a loam classified as a Haplic Alisol (FAO 1988). Some important soil chemical properties are showed in Table 2.
Surveys have showed that the bedrock, soil, vegetation and climate are uniform throughout the watershed.

Forest management in the watershed is limited and selective thinning has taken place every 10 years, for at least the last 100 years (information obtained from local Forest Service). The oldest trees, making up 10–12% of the total wood volume in the watershed, are removed at harvesting. In 2003, the stem wood volume (including bark) was 62 m$^3$ ha$^{-1}$. The last time that thinning took place was in 2000.

### 2.2 Precipitation and Stream Water Sampling

Bulk deposition was collected in a nearby forest clearing using two polyethylene funnels having a diameter of 18 cm, connected to a five-litter polyethylene bottle through a 1.05 m PVC pipe having a diameter of 20 cm. Throughfall was collected using 10 collectors, identical to those for bulk precipitation, placed randomly in the beech stand. For snow sampling during the winter months (November to April), the collecting funnels and bottles were replaced with appropriate plastic bags, having approximately 90 cm length, fitted firmly inside the PVC pipes. Stemflow was...
collected from two trees having an average breast diameter of the forest stand by means of a polyethylene tube split into half and wound firmly around the stem of each tree. Stream water discharge was measured at the outlet of the watershed (where the plot was located) with a Cipolletti weir, equipped with a water level recorder, and sampled just upstream from the weir.

Water collection was done on a weekly basis apart from stream water, which was done twice a month. Other details of rainfall measurements in the plot are described by Michopoulos et al. (2001).

2.3 Forest Floor and Mineral Soil Sampling

Forest floor (litter plus humus layer) and mineral soil were collected in August of 2002 by systematic sampling. For forest floor collection an iron template of $15 \times 15$ cm was used. Separate sampling was done for the Oh soil horizon to determine pH, C and N concentration and the C/N ratio. Mineral soil was taken at three depths, 0–20, 20–40 and 40–80 cm with an auger having a diameter of 15 cm. The number of samples of forest floor, Oh horizon and mineral soil was 12 for each category.

Bulk density of mineral soils was measured by collecting undisturbed soil cores from a pit (three replicates for each depth layer) by means of a $195 \text{ cm}^3$ cylinder at the three mentioned depths (0–20, 20–40 and 40–80 cm). The volume percentage occupied by stones was estimated visually and was taken into account when calculating total soil (<2 mm) mass. The bulk density of fine earth material was used to calculate N pools in mineral soils.

2.4 Litterfall and Leaf Collection

For litterfall collection, 10 littertraps in the form of cylindrical plastic buckets, each having a collecting area of 0.242 m$^2$, were placed systematically along a line in the plot, approximately 0.50 m above ground at a distance of 10 m from each other. The bottom of each bucket was perforated so that rain or snowmelt water could drain out. A plastic net was put at the bottom of the littertraps to avoid loss of small material. Collection of litterfall was done monthly or for longer periods, depending on the accessibility of the area due to snow. A composite sample was formed and transported to the laboratory for analysis. No separation into leaves, branches, fruits and other fractions of litterfall were made.

Leaves were cut from the upper part of tree crowns by means of shooting. For this purpose, five dominant beech trees were selected in the stand. Leaf collection started in 1995 and was done every two years in August from the same trees. After each collection, a composite sample was formed. This sample was dried at 80°C for 48 h and ground in a stainless blade mill.

2.5 Biomass and Increment Measurements

The breast diameter and height of all trees were measured in the plot area of 0.273 ha in the autumn of 1996, 2000 and 2003. In total, 115 trees were measured. Allometric equations (Apatidis and Sifakis 1999) specific for the European beech, taking into account trunk diameter and tree height, were applied to estimate the bark and wood volumes of each tree in the year 2003. For the year 2002, average diameters and heights for each tree were calculated from the difference between measurements in the years 2000 and 2003 (for each tree). For each year, the bark and wood volumes of the 115 trees were added to find the total volumes in the plot area and in the area of one ha. The branch volume for each tree was estimated visually as a percentage of the trunk tree volume. The estimation was done by experienced foresters on assessment of tree health condition and phenology. Stem and branch biomass was calculated in the years 2002 and 2003 by taking into account wood and bark density. For this, a tree having the average diameter in the plot was cut down and sub-samples of wood and bark were taken for density measurements and chemical analysis. In order to calculate the wood and bark density, the volumes of the wood and bark sub-samples were measured immediately by immersing the samples into a volumetric cylinder filled with deionized water and then weighed after oven drying at 80°C for 48 h. With the knowledge of total wood and bark volume, their density and N concentrations,
the amounts of N in wood and bark per ha were calculated.

Live leaf biomass was calculated using the allometric equations for mature even aged European beech stands reported by Kittredge (1944). These equations use tree diameter and yield the green (fresh) foliage weight. In order to convert the N amounts in foliage to dry weight, sub-samples of beech leaves were selected from the same trees from which leaves were taken for chemical analysis and dried at 80°C for 48 h.

Tree stand N requirements (kg ha\(^{-1}\) yr\(^{-1}\)) for the two hydrological years were estimated by adding the amounts of N in live leaf biomass and the use for annual stem and branch annual increment (including bark). Tree uptake of N was calculated by summing up the N amounts in litterfall and annual increment of trunk and branches. The difference between N requirement and uptake is the amount of N retranslocated (Cole and Rapp 1981).

2.6 Sample Pretreatment and Chemical Analysis

The volume of water samples was measured and samples stored immediately in a fringe at 4°C for a maximum of two weeks. If longer storage periods were needed, the samples were then stored at –2°C. Prior to ion chromatography analysis, all samples were filtered through a 0.45 µm membrane filter.

The Oh horizon and mineral soil samples were air-dried for a week and passed through a 2 mm sieve. They were then dried at a temperature of 80°C for 48 h and analyzed for total N. Forest floor samples collected by the iron template were dried separately, weighed and pulverized (without sieving) in a ball mill.

The litterfall (composite), wood and bark samples were dried at 80°C for 48 hr, weighed and pulverized in a ball mill.

Concentrations of ammonium and nitrate N in waters were determined by ion chromatography. All the criteria for analysis defined by EMEP (1996) were applied.

Concentrations of total N in forest floor, mineral soil, litterfall, leaf, wood and bark samples were measured by the Kjeldahl distillation method. Total P in ground leaf samples was determined after igniting at 480°C for six hours and dissolving the ash in 20% HCl. Phosphorus concentrations were measured in the digests by means of the ammonium-molybdate-method in a UV spectrophotometer.

The pH of the Oh horizon and mineral soils was measured electrometrically in a suspension of soil and water (1:2.5 for the Oh horizon and 1:1 (w/v) for mineral soils).

Organic C in the Oh horizon and mineral soils was determined by oxidizing the organic C with K\(_2\)Cr\(_2\)O\(_7\) (Walkley 1946).

2.7 Data Handling and Statistical Analysis

All results refer to hydrological years (1st October–30th September) so that N inputs can be associated with N outputs.

For each month, the volume-weighted mean concentrations of ammonium and nitrate N in mg L\(^{-1}\) were calculated for bulk deposition, throughfall, stemflow and stream water. The fluxes (kg ha\(^{-1}\) yr\(^{-1}\)) of inorganic N for bulk deposition and throughfall were calculated taking into account N concentrations, water volumes and area of the collecting funnels. The fluxes of N for stemflow were calculated using N concentrations, water volumes (produced by stemflow from all trees in the plot) and the total area of the plot, whereas for stream water the N fluxes were calculated using N concentrations and water volumes per area of discharge recorded at the hydrometric station.

All concentrations of N in soils, leaves and litterfall are expressed on an oven dry-basis (80°C). Nitrogen pools were calculated for forest floor plus 0–80 cm soil layer.

Parametric statistics (paired t test for the same periods) were used to test for significant differences in litterfall masses and N fluxes in litterfall in the two hydrological years. Concentrations of ammonium N in stream water in the first year were compared with the concentrations of ammonium N in the second year for the same period (month) and nitrate N concentrations in stream water in the first year were compared with the concentrations of nitrate N in the second year for the same period (month) using nonparametric statistics (Wilcoxon paired T-test). Nonparametric
tests, being distribution free, are more appropriate than parametric tests with regard to hydrological data (Hirsh and Slack 1984).

Parametric statistics were applied to find possible significant trends in the N/P ratios of foliage. Although the number of observations was not high, it is known from experience that litterfall amounts and nutrient concentrations follow normal distributions. The serial randomness of measurements of the ratios was tested by the mean square successive difference test (Zar 1996).

3 Results
3.1 Nitrogen Concentrations and Fluxes in the Various Water Phases

Bulk precipitation was 14.9% lower in the first hydrological year than the mean annual value (1,626 mm), whereas it was 46.5% higher in the second year (Table 3).

Crown interception (%), \[\frac{\text{Bulk} - (\text{Throughfall} + \text{Stemflow})}{\text{Bulk}} \times 100\], was 10.4 and 4.8% for the two respective years calculated from the accumulated values at the end of each year. These values are relatively small for mature beech forests, which can be attributed to fog precipitation. The mean annual amount of fog precipitation was estimated to be 14.2% of the corresponding mean annual bulk deposition (Baloutsos et al. 2004). Without adding the amount of fog precipitation to the amount of gross precipitation, the percentage of interception is underestimated and that of throughfall and stemflow overestimated. This additional component, however, affects only the percentages of the components in the water balance equation and not the concentrations of chemical parameters of precipitation for the reason that fog precipitation cannot settle on the bulk deposition collectors.

<table>
<thead>
<tr>
<th>Table 3. Components of bulk deposition (mm) in the two hydrological years.</th>
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<td>---------------------------------------------------------------</td>
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<tr>
<td>Bulk</td>
</tr>
<tr>
<td>Throughfall</td>
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<tr>
<td>Stemflow</td>
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<td>Stream water</td>
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<td>Interception</td>
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Table 4. Median values of volume weighted monthly concentrations (mg L\(^{-1}\)) of inorganic N in precipitation phases during the two hydrological years. ( ) = range.

<table>
<thead>
<tr>
<th></th>
<th>(\text{NH}_4^+\text{-N})</th>
<th>(\text{NO}_3^-\text{-N})</th>
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<tbody>
<tr>
<td>Bulk</td>
<td>0.341</td>
<td>0.474</td>
</tr>
<tr>
<td></td>
<td>(0.19–1.03)</td>
<td>(0.09–1.24)</td>
</tr>
<tr>
<td>Throughfall</td>
<td>0.442</td>
<td>0.687</td>
</tr>
<tr>
<td></td>
<td>(0.17–1.07)</td>
<td>(0.04–4.79)</td>
</tr>
<tr>
<td>Stemflow</td>
<td>0.382</td>
<td>0.501</td>
</tr>
<tr>
<td></td>
<td>(0.02–6.46)</td>
<td>(0.02–6.46)</td>
</tr>
<tr>
<td>Stream water</td>
<td>0.040</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>(0.01–0.23)</td>
<td>(0.02–0.48)</td>
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Table 5. Fluxes of inorganic N (kg ha\(^{-1}\)) in precipitation phases during the two hydrological years.

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<th>(\text{NH}_4^+\text{-N})</th>
<th>(\text{NO}_3^-\text{-N})</th>
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<tbody>
<tr>
<td>Bulk</td>
<td>5.92</td>
<td>7.68</td>
</tr>
<tr>
<td>Throughfall</td>
<td>5.16</td>
<td>6.36</td>
</tr>
<tr>
<td>Stemflow</td>
<td>0.29</td>
<td>0.43</td>
</tr>
<tr>
<td>Stream water</td>
<td>0.29</td>
<td>0.94</td>
</tr>
</tbody>
</table>
Considering that stemflow is part of throughfall, enrichment was found for both medians of ammonium and nitrate N concentrations beneath the beech canopy with regard to bulk deposition (Table 4). However, the bulk deposition fluxes for ammonium N were higher than the sum of throughfall and stemflow fluxes for the ammonium N (Table ).

The ammonium and nitrate N concentration in stream water was about 10 times lower than in other water phases (Table 4) with the exception of nitrate N in 2002–2003. When all N inputs to the forest floor in the form of throughfall and stemflow and outputs in the stream water were considered from Table 5, a net gain for N was found for the forest floor and mineral soil, i.e. 11. and 12.2 kg ha$^{-1}$ yr$^{-1}$ for the year 2001–2002 and 2002–2003, respectively.

### 3.2 Nitrogen Amounts and Dynamics in the Beech Ecosystem

The average concentrations of N and P in foliage derived from the sampling years 1995, 1997, 1999, 2001 and 2003 were 21.7 and 1.09 g kg$^{-1}$, respectively. The coefficient of variation was low for N (6.7%) and rather high for P (17.8%). The average value of the ratio N/P was 20.4, with a coefficient of variation 15.5%.

The mass of litterfall produced in the year 2001–2002 was 3.43 Mg ha$^{-1}$ and 5.63 Mg ha$^{-1}$ in the year 2002–2003. The difference in the litterfall production (paired t-test for the same periods) between the two years was significant (p<0.05). The amount of N stored in the various compartments of the ecosystem decreased in the order: mineral soil > above ground biomass > forest floor, whereas N requirements exceeded N uptake (Table 6). From this Table the N amount retranslocated, for each year, can be found by subtracting the amount of N uptake from the amount of N requirement. Thus for the hydrological year 2001–2002 51.1% and 48.9% of the annual requirement of N were met through uptake and retranslocation, respectively, whereas for the 2002–2003 year the corresponding percentages were 65.5 and 34.5%. Also from Tables 5 and 6 it can be found that the total amount of inorganic N in bulk deposition provides 21.6 and 20.1% of the annual N uptake for the hydrological years 2001–2002 and 2002–2003, respectively.

### 4 Discussion

#### 4.1 Nitrogen Concentrations and Fluxes in Deposition

The non-existence of data for dissolved organic N in the various water phases is a shortcoming of this work. Organic N can make a substantial percentage of the total dissolved N. Especially, for stream water organic N can contribute 60% of the total dissolved N (McHale et al. 2000). Most literature refers to dissolved inorganic N but nowadays the determination of organic N is
gaining ground.

Nitrogen concentrations in bulk, throughfall and stemflow were not high according to literature for beech forests (Chang and Matzner 2000). This is due to the dilution caused by the high rainfall amounts, generally observed in the area. Chang and Matzner (2000) found almost twice the concentration of both ammonium and nitrate N in throughfall and stemflow in a beech forest in Germany but with an annual precipitation of 750 mm.

The fluxes of total inorganic N in bulk deposition were 10.7 and 13.6 kg ha\(^{-1}\) yr\(^{-1}\) for the first and second hydrological year, respectively. These values are close to the mean N input value of 13.1 kg ha\(^{-1}\) yr\(^{-1}\) for 309 forest sites across Europe (UN-EC 2001).

In both years, net throughfall fluxes for N (calculated as the difference between throughfall plus stemflow and bulk deposition fluxes) showed a small retention of ammonium N in the canopy, (0.47 and 0.89 kg ha\(^{-1}\) for the hydrological years 2001–2002 and 2002–2003, respectively), and a release of nitrate N (2.15 and 1.84 kg ha\(^{-1}\) in the first and second year, respectively). Although uptake of N by tree canopies, especially for ammonium N, has been shown (Draaijers et al. 1997), our beech forest did not show significant canopy uptake of N. The considerable enrichment of nitrate N beneath the tree canopy can be attributed to the washing off of dry deposition consisting of HNO\(_3\) vapor and particles containing nitrate salts (Lovett and Lindberg 1993). In this work, total (wet plus dry) N deposition was not calculated and therefore the net canopy exchange for N cannot be estimated. However, the net throughfall fluxes can be considered as an indication of ammonium N consumption and nitrate N release. In both years, the fluxes of inorganic N in throughfall plus stemflow exceeded the limit of 10 kg ha\(^{-1}\) yr\(^{-1}\) reported in literature (Dise and Wright 1995) as the threshold for triggering N leaching. However, the median concentrations of nitrate N in stream water remained low. As will be shown in next section, there was an appreciable difference between N requirement and uptake indicating considerable N deficiency, which is why there was retention and minimal leaching losses, i.e. N saturation not reached:

4.2 Nitrogen Amounts and Cycling in Aboveground Biomass

The authors stress the issue that the belowground "litterfall" and increment were not measured. Since the annual turnover of fine roots is very high in forested ecosystems (Vogt et al. 1986), our estimates of both N uptake and requirements are underestimates.

The N requirement of the beech forest in both years is close to the average value (110 kg ha\(^{-1}\) yr\(^{-1}\)) reported by Cole and Rapp (1981) for beech stands in Germany. For most deciduous trees, N requirements cannot be met by N uptake from the soil and use is made of N retranslocated back from the leaves before abscission. Some N requirement can be met by uptake of NH\(_4\) and NO\(_3\) from wet deposition. Brumme et al. (1992) sprayed young beech trees with \(^{15}\)N and found that 6% of the total N in leaves was derived from the sprayed N. Cole and Rapp (1981) quote an average N uptake for beech stands of 66.5 kg ha\(^{-1}\) yr\(^{-1}\). This value is close to the uptake value we found in the hydrological year 2002–2003 and a little higher than that in the first hydrological year. However, there is a subtle difference. In our beech stand, the N amounts immobilized in the annual increment were twice as high as the average increment values (10 kg ha\(^{-1}\) yr\(^{-1}\)) quoted by Cole and Rapp (1981). The N trapped in the increment cannot be recycled for a long time, with the beech ecosystem functioning as a sink for N. In one mature beech forest in Denmark, Beier et al. (2001) calculated an annual N uptake of 14 kg ha\(^{-1}\). It must be pointed out that, so far, no author has made clear if bark had been analyzed for N before calculating the amount of N immobilized in annual increments. The differences in N concentrations in wood and bark tissue are quite high. In this work, it was found that N concentrations in wood and bark tissue were 1.5 and 4.8 g kg\(^{-1}\) respectively. Although the bark yields little mass compared to wood, the N amount in bark can be significant. In this study, the N amount in bark made up approximately 18% of the total N amount in trunks and branches, whereas the N amount in the bark increment was approximately 27% of the wood increment (average of the two years).

Litterfall production was significantly higher in
the wetter hydrological year 2002–2003 and for this reason the N uptake was also higher. Annual litterfall mass variation has been attributed to soil water availability (Regina and Tarazona 2001). Increased litterfall production in the second year can also be the result of milder temperatures in the first year, especially during spring. Contrary to this study, Pedersen and Bille-Hansen (1999) found that annual litterfall production was independent of precipitation in a beech forest stand in Denmark. Despite the significant differences in litterfall production, N returns to the forest floor through litterfall did not differ in the two hydrological years. This is another way of conserving N when litterfall production fluctuates in time.

Another indication that N saturation was not reached was that the ratio of N/P in foliage did not change significantly from year to year. According to Aber et al. (1998), foliar analysis is probably the best indicator of ecosystem N status and degree of N saturation. These authors argue that the process of N saturation is controlled mainly by plant uptake rather than by soil microbial uptake so plant processes would be the first to change in response to N additions.

4.3 Nitrogen in Forest Floor and Mineral Soil

The N pool of the forest floor is average but that of mineral soil was not high compared to values reported by Cole and Rapp (1981) in forest floors (86 to 1050 kg·ha⁻¹) and mineral soils under beech down to rooting zone (6332 to 9452 kg·ha⁻¹). Nevertheless, our soil N pool exceeded the threshold of 5000 kg·ha⁻¹ that Cole et al. (1992) set for a forest soil having a C/N < 20 to be considered susceptible to N leaching. The C/N ratio in the Oh horizon was 15, which is less than 25 used to assess the risk of nitrate leaching (Macdonald et al. 2002).

4.4 Nitrogen in Stream Water

With regard to results found by other workers for undisturbed watersheds, the ammonium N concentration is higher but that of nitrate N concentration is low. Nitrate leaching only occurs when forests are disturbed (other than if N saturated). In a number of undisturbed watersheds of the Hubbard Brook experimental forest, the ammonium N concentration was 0.031 mg L⁻¹ and that of nitrate N 0.44 mg L⁻¹ (Likens and Borman 1995).

The concentrations of nitrate N and ammonium N in stream water did not differ significantly from each other in either of the two hydrological years. The N retention capacity of the ecosystem is manifested by the positive difference between throughfall plus stemflow and stream water nitrate and ammonium fluxes. In general, the ammonium ion is retained more easily than the nitrate by forest soils through abiotic fixation and assimilation by micro-organisms (Myrold and Tiedje 1986). Furthermore, European beech species take up more ammonium N than nitrate N (Gessler et al. 1998). Nitrate immobilization in soil has also taken place in the beech forest. Although nitrification was not measured in this work, it is probable that the higher flux of inorganic N in bulk deposition in the second year (2.9 kg ha⁻¹ yr⁻¹) resulted in increased nitrification rates. Månsson and Falkengren-Gerup (2003) found that 17 kg ha⁻¹ yr⁻¹ of inorganic N in bulk deposition caused five times as much nitrification in the Oh horizons of deciduous forests in Sweden as 10 kg ha⁻¹ yr⁻¹. The average nitrate N leaching flux in European beech forests is 4.1 kg ha⁻¹ yr⁻¹ (Eichhorn et al. 2001), whereas our beech forest showed a maximum flux of nitrate N losses of 1.46 kg ha⁻¹ yr⁻¹ in the year 2002–2003. The effect of low soil pH (like that in our beech plot) on nitrification is controversial. Robertson (1982) found that net nitrification was absent from 20% of the acid soils (pH < 5) in deciduous forests. Some authors accept that autotrophic nitrification does occur in acid forest soils (De Boer and Kowalchuk, 2001). The same authors support that heterotrophs also make some contribution to nitrification in acid soils. The pH of the soils mentioned by the authors Månsson and Falkengren-Gerup (2003) was low (3.5–4.0), another indication that nitrification is present in acid forest soils.

Nitrate in soils has been shown to be immobilized by soil microbes (Davidson et al. 1992), mycorrhizae (Aber et al. 1998), and abiotic transformation of nitrates into soluble organic compounds (Dail et al. 2001). It is probable that all these processes take place at the same time. In some mountain beech forests, high con-
centrations of nitrates are found in stream water during snowmelt, the reason being the start of N mineralization before leaf flush and uptake (Rothe et al. 2002). As snowmelt started in February in the first year and in April in the second year, it would be expected that concentrations of nitrate N would be higher in the second year. It is probable that in Mediterranean mountainous forests, soil microbial activity starts a little later than other mountain forests in central Europe because of high temperature in late spring.

5 Conclusions

The presented hypothesis in the Introduction section is rejected. The beech forest displaced an appreciable capacity to retain N. This capacity can be attributed to several factors. One is the N deficiency manifested by the large difference between N uptake and requirements. Low nitrification rates because of low soil pH cannot be excluded.

Acknowledgements

The authors express their sincere thanks to the Greek Ministry of Agricultural Development and Food as well as the European Union, which funded this research. They are also indebted to M. Voulala and A. Bourletsikas for their help with data handling and H. Mitropoulou for her help with analytical work.

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