

## Chapter 3

# **NUTRIENT TURNOVER, GREENHOUSE GAS EXCHANGE AND BIODIVERSITY IN NATURAL FORESTS OF CENTRAL EUROPE**

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## **ABSTRACT**

We measured the microbial turnover of carbon (C) and nitrogen (N) in 12 natural forest reserves in Austria, along with estimating potential emission rates of nitrous oxide (N<sub>2</sub>O) and carbon dioxide (CO<sub>2</sub>), and uptake rates of methane (CH<sub>4</sub>). The community composition of soil microorganisms was investigated using PLFA (Phospholipid fatty acid) analysis and molecular tools. The biodiversity of selected taxa of micro-, meso- and macrofauna were studied. The aim was to provide a reference data set for the evaluation of the soil biology in managed, especially disturbed or damaged forest ecosystems. Ecophysiological quotients were tested for their ability to make predictions about the carbon dynamics of forest soils. The 12 forests represented the six typical forest types in Central Europe: oak, beech, spruce-fir-beech, floodplain, and pine forests. Nitrogen turnover rates were high in soils with sufficient soil moisture and high pH. Nitrogen losses as nitrate or N<sub>2</sub>O were small unless N deposition was as high as 30 kg ha<sup>-1</sup> yr<sup>-1</sup>. The fastest turnover of C and N occurred in the floodplain forests, based on microbial quotients, xylanase activity, the relative thickness of litter layer and <sup>15</sup>N abundance in the organic soil. Carbon turnover was slowest in the beech forests on acidic bedrock, and slow turnover may lead to the largest net C accumulation. Tree species had distinct effects on microbial community composition, but the high soil biodiversity in these natural forests may not be greater than in managed forests.

From: Binkley, D., and O. Menyailo (eds). 2005. Tree Species Effects on Soils: Implications for Global Change. NATO Science Series, Kluwer Academic Publishers, Dordrecht.

## INTRODUCTION

Almost 50% of the total area of Austria is forested, and the forests are dominated by commercially valuable stands of Norway spruce (*Picea abies*). The few remaining forests that resemble the natural vegetation composition are located in forest reserves with restricted management. These natural forests are used as reference systems for evaluating silvicultural research on sustainable forest management. Natural forests are expected to have high biodiversity, where the structural richness of the habitat enables complex relationships between fauna, flora, and microflora. They also provide refugia for rare plants and animals found only in natural forest types. Austria had 180 of these forest reserves up to the year 2003. Most of these forests are privately owned, and owners are compensated by the government for loss of income associated with conservation status. The Ministerial Conference for the Protection of Forest Ecosystems (MCPFE) has launched a world-wide network of protected forest areas which should cover all major forest types (MCPFE and UNECE/FAO, 2003).

The sites selected for our investigation of soil conditions and communities were chosen by vegetation ecologists and soil scientists. The stands have developed under natural competition conditions with no management interventions. All sites were well documented with known forest history. Our set of sites spans gradients of environmental conditions as well as species composition, providing a realistic evaluation of the interactions of biotic and abiotic factors. This approach complements the “common garden” experiments, where the effects of tree species are compared in isolation from variations in environmental conditions. All investigated sites were within an area of 200 km in eastern Austria, representing a variety of vegetation types from subpannonic lowlands to the Bohemian massif.

Microbial nutrient turnover and greenhouse gas exchange of the forests provide key information about interactions between soils and trees. Soils support trees, and trees supply the growth substrate to the microbial populations in the soil via litter and root exudates. The microbial population developing under a certain forest type feeds back on tree growth via decomposition, nutrient release and immobilisation processes. Microbes mediate the turnover of greenhouse gases in the soil and hence determine whether a forest constitutes a net source or a net sink for greenhouse gases.

This chapter provides an overview of several studies of natural forests in Austria. We include some previously unpublished data, and draw conclusions in the context of tree and soil interactions and global climate change.

## MATERIAL AND METHODS

### Study sites and sampling design

Twelve natural forest stands were selected within the eastern part of Austria, featuring oak-hornbeam, woodruff-beech, acidic beech (beech forests on siliceous bedrocks with acidophilous ground vegetation), spruce-fir-beech, floodplain and Austrian pine forests. All stands were old growth forests, characterized by a natural tree species composition. An overview of the site characteristics and the geographical locations is given in Table 1. For the microbiological analyses, soils from each stand were sampled in spring and autumn of two successive years (Hackl et al., 2000; Hackl et al., 2004a) and for the DNA analysis another autumn sampling was performed (Hackl et al., 2004b).

### Soil chemical and microbiological analyses

The soil samples were stored at  $-20\text{ }^{\circ}\text{C}$  and prior to analysis were sieved to 2 mm. Soil pH was measured in  $\text{CaCl}_2$ -solution by glass electrode (soil: 0.01 M

*Table 1.* Site characteristics and soil chemical properties of 12 natural forest stands (data from Hackl 2004c). Soil chemical data represent means from 10 subsamples  $\pm$  standard error.

Forest type	Site	Elevation (m)	Temperature ( $^{\circ}\text{C}$ )	Precipitation (mm)	Soil type	Geology	Organic C (%)	Total N (%)	C:N	pH
Oak-hornbeam	JE	325	8.8	643	Dystric Planosol	Sandstone/Claystone	5.04 $\pm$ 0.34	0.22 $\pm$ 0.01	23.4 $\pm$ 0.6	4.5 $\pm$ 0.2
	K	270	8.7	593	Calcaric Planosol	Micashist/Limestone	4.23 $\pm$ 0.21	0.20 $\pm$ 0.01	21.0 $\pm$ 0.4	5.4 $\pm$ 0.3
Woodruff-beech	JB	320	8.8	643	Dystric Planosol	Sandstone/Claystone	4.38 $\pm$ 0.31	0.19 $\pm$ 0.01	22.5 $\pm$ 0.8	5.1 $\pm$ 0.3
	KI	510	7.6	768	Dystric Cambisol	Sandstone/Claystone	4.36 $\pm$ 0.32	0.33 $\pm$ 0.02	13.1 $\pm$ 0.4	4.1 $\pm$ 0.0
Acidic beech	D	500	7.6	613	Dystric Cambisol	Gföhl gneiss	9.45 $\pm$ 0.34	0.35 $\pm$ 0.04	26.9 $\pm$ 0.7	4.6 $\pm$ 0.2
	S	550	7.4	631	Dystric Cambisol	Gföhl gneiss	7.03 $\pm$ 0.56	0.30 $\pm$ 0.02	23.5 $\pm$ 0.5	4.0 $\pm$ 0.0
Spruce-fir-beech	R	1035	5.5	1759	Chromic Cambisol	Dolomite	16.00 $\pm$ 2.38	0.94 $\pm$ 0.15	17.1 $\pm$ 1.1	4.9 $\pm$ 0.4
	N	995	5.8	1262	Stagnic Luvisol	Sandstone	6.46 $\pm$ 0.63	0.38 $\pm$ 0.03	16.9 $\pm$ 0.7	4.0 $\pm$ 0.1
Floodplain	M	160	9.7	582	Calcaric Fluvisol	Recent clay	5.46 $\pm$ 0.34	0.47 $\pm$ 0.02	11.7 $\pm$ 0.4	7.2 $\pm$ 0.0
	B	160	9.7	534	Calcaric Fluvisol	Recent clay	3.92 $\pm$ 0.13	0.23 $\pm$ 0.01	17.2 $\pm$ 0.5	7.4 $\pm$ 0.0
Austrian pine	St	640	7.0	668	Rendzic Leptosol	Dolomite	16.99 $\pm$ 2.69	0.61 $\pm$ 0.09	28.0 $\pm$ 1.0	7.4 $\pm$ 0.1
	Me	475	8.2	554	Rendzic Leptosol	Dolomite	9.64 $\pm$ 0.63	0.26 $\pm$ 0.02	37.0 $\pm$ 2.0	7.4 $\pm$ 0.0

CaCl<sub>2</sub> = 1:5), and total soil carbon (C<sub>t</sub>) and nitrogen (N<sub>t</sub>) concentrations were analysed after dry combustion. Soil samples were weighed into tin capsules for isotope ratio mass spectrometry (IRMS); the continuous-flow IRMS system consisted of an elemental analyser interfaced to the gas isotope ratio mass spectrometer (Delta Plus, Finnigan MAT). Reference gas was calibrated to the atmospheric N<sub>2</sub> standard using IAEA (International Atomic Energy Agency, Vienna, Austria) reference material. The standard deviation of repeated measurements of a laboratory standard was 0.15‰ for δ<sup>15</sup>N (Wanek and Arndt, 2002).

Extractable ammonium (NH<sub>4</sub><sup>+</sup>-N) and nitrate (NO<sub>3</sub><sup>-</sup>-N) were determined in 2 M KCl-extracts as described in Hackl et al. (2004a). Low molecular weight organic compounds of the soil organic matter (sugars, amino acids) were analyzed in extracts of 60% acetone according to Hackl et al. (2000).

Nitrogen mineralization potential and urease activity were measured according to Kandeler (1996a,b). Microbial biomass was determined as ninhydrin-reactive N by a chloroform fumigation-extraction technique and was also measured by substrate-induced respiration (SIR) with glucose-amendment (Hackl et al., 2000). From SIR-data the microbial quotient as well as the metabolic quotient were calculated according to Insam (1996).

Nitrate translocation into deeper soil horizons was measured by applying a soil core-IER method (Binkley and Hart 1989). Resin bags containing ion exchange resins (IER, 4 mg Dowex 1X8 pract. and 4 mg Amberlite IR 120 pract.) were placed under soil cores (10.5 cm depth, 9 cm diameter) which were taken from each sampling point. The resin bags and the soil cores were enclosed in close-fitting PVC tubes and returned to the same holes from which the cores had been taken. Two months later the resin bags were removed and extracted two-fold with 1.59 M HCl. After neutralisation with NaOH, NO<sub>3</sub><sup>-</sup>-N was measured colorimetrically as NO<sub>2</sub><sup>-</sup>-N after reduction with copper sheathed granulated zinc (Kandeler 1996d).

## Gas flux measurements

Nitrous oxide (N<sub>2</sub>O) and carbon dioxide (CO<sub>2</sub>) production and methane (CH<sub>4</sub>) degradation were measured by incubating intact soil cores, which had been stored at 4 °C for not more than 4 days prior to analysis. Each soil core was enclosed in a 500 ml gas-tight glass-jar and kept at 25 °C for 24 hours in 1997 and for 6 hours in 1998, after ensuring that increases in concentration were linear. At the beginning and at the end of the incubation period gas samples were taken. Head space air of the vessels was transferred into evacuated 10 ml glass vials with a gas-tight syringe, and stored under water until analysis. Gas samples were analyzed by gas chromatography using a <sup>63</sup>Ni electron capture detector for N<sub>2</sub>O (injector 120 °C, detector 330 °C, oven 30°C, carrier gas N<sub>2</sub>; Zechmeister-Boltenstern, 1994), TCD for CO<sub>2</sub> (injector 120 °C, detector 150 °C, oven 80°C, carrier gas Helium) and a FID for CH<sub>4</sub> (Rigler and Zechmeister-Boltenstern, 1999).

Microbial communities were analyzed by phospholipid fatty acid (PLFA) extraction and analysis was done as described by Frostegård et al. (1996), with some modifications as described by (Hackl et al., 2004b). Bacterial communities in the forest soils were also analyzed by extracting bacterial community DNA from soil samples. After PCR amplification with 16S rRNA gene primers, amplicons were subjected to terminal restriction fragment length polymorphism (T-RFLP) analysis (Hackl et al., 2004b). For selected forest sites clone libraries were constructed using the same DNA preparations as for T-RFLP analysis (Hackl et al., 2004b).

### **Statistical analysis**

The SAS System for Windows, Version 8 was used for statistical processing of data. Analysis of variance and Tukey test were applied to test for significant differences in ecophysiological parameters among forest types. Correlations between N<sub>2</sub>O production rates and soil chemical and microbiological variables were determined using Spearman's rank correlations. Stepwise linear regression analyses were used to explore the relationships between nitrogen transformation rates. Cluster analysis of PLFAs were conducted using the program S-Plus after checking normal distribution of data. The average linkage procedure was selected. SPSS was used for the creation of Chernoff faces, which is a method of visualizing similarities and differences among multidimensional data. The program displays faces that are described by 15 facial characteristic parameters such as head eccentricity, eye eccentricity, pupil size, nose size, mouth shape, eye spacing, eye size, and mouth length.

## **RESULTS AND DISCUSSION**

### **Nitrogen turnover**

Plant roots release low molecular weight organic compounds, such as sugars and amino acids, which serve as carbon and nitrogen sources for microorganisms. The concentrations of 15 different amino acids in 12 natural forest soils are illustrated in Figure 1. Each parameter is represented by a number, namely the concentration of an amino acid, which is designed as a feature of the faces shown. For example the length of the nose indicates the amount of the amino acid glycine, whereas the shape of the mouth is determined by the amino acid citrullin. The figure shows forests with similar amino acid composition. One forest stands out from the others, with a very different and well balanced amino acid composition: the spruce-fir-beech forest. This forest is called "Urwald Rothwald", and has never been managed in historical times. Microbial biomass and the concentration of sugar were

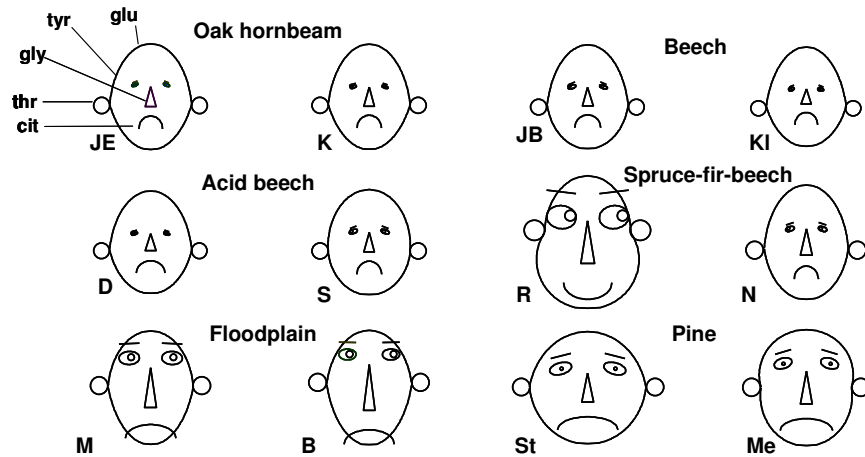


Figure 1: Chernoff faces reflecting concentrations of 15 amino acids (data from Hackl, 2000). Face breadth = aspartic acid, ear position = threonine, face length = serine, upper face eccentricity = glutamic acid, lower face eccentricity = proline, nose length = glycine, mouth center = alanine, mouth shape = citrulline, mouth width = valine, eye level = methionine, eye spacing = isoleucine, eye tilt = leucine, eye eccentricity = tyrosine, eye length = phenylalanine, pupil position = sum of amino acids.

twice the amounts of other soils (Hackl et al., 2000). This forest is situated in a remote valley in the limestone Alps and receives annually almost 1800 mm precipitation. The faces in Figure 1 show similarities in amino acid composition among the oak-hornbeam, beech and acidic beech forests, with high concentrations of leucine, serine and glutamine. In the pine forests traces of proline, methionine and tyrosine could be detected, which were not found in the other soils. These amino acids are produced by plants as osmoregulators in order to protect them from drought stress.

Three aspects of N-turnover are depicted in Figure 2: potential N-mineralization, N-storage in the microbial biomass, and activity of urease (an enzyme that releases ammonium ( $\text{NH}_4^+$ )). All three variables correlated positively with total soil N ( $p < 0.05$ ). Microbial biomass could be calculated from N mineralization potential and total soil N ( $r^2 = 0.730$ ), urease activity was a function of N mineralization potential and total soil N ( $r^2 = 0.653$ ) (Hackl et al., 2004). Turnover rates of N varied across forest types, partially in response to soil moisture and pH. Similar relationships have been found in forest soils close to New York (Templer et al., 2003). The highest rates of N turnover were measured in soils of the virgin forest “Rothwald”, the virgin spruce-fir-beech forest that had the particular amino acid composition in Figure 1. The lowest N-turnover was measured in an acidic beech forest on silicate bedrock. As can be seen in Figure 3 total N, microbial N and extractable mineral N in the spruce-fir-beech forest “Rothwald” exceeded concentrations in the acidic beech forest “Saubrunn” by three- to fourfold. The ratio of nitrate to ammonium was very high (1:477) in the acidic beech

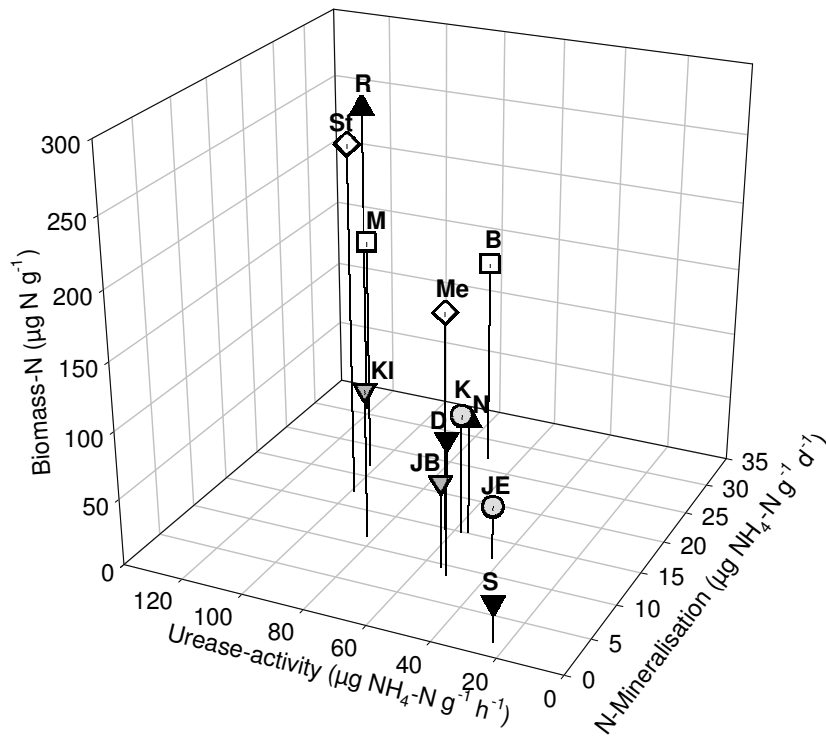


Figure 2: Potential nitrogen turnover at 12 natural forest sites (data from Hackl et al., 2004a).

forest, and moderate in the spruce-fir-beech forest with 1:5. Mineralisation rates differed strongly, whereas  $N_2O$ -emissions were low in both forests. Although N-turnover rates differed greatly between these forests, neither seemed prone to high losses of  $N_2O$  or nitrate, and both forests showed a fairly closed N-cycle. Other forests, including the floodplain forests, had slower but less closed N-cycles. This could be seen from intermediate N turnover rates but comparably high  $N_2O$  and nitrate leaching values (Hackl et al., 2004a; Zechmeister-Boltenstern et al., 2000).

### Net greenhouse gas exchange

Carbon dioxide exchange of forests has received much attention although the soil compartment is still not fully understood (Giardina and Ryan, 2000; Trumbore et al., 1996; Valentini et al., 2000). Even less is known about nitrous oxide and methane turnover which can significantly affect the net greenhouse gas exchange. According to the Third Assessment Report of the IPCC (Intergovernmental Panel on Global Climate Change), one molecule of methane has the global warming potential of 23 molecules of  $CO_2$ , and one molecule of  $N_2O$  counts as 296 equivalents of  $CO_2$  (IPCC, 2003). We found highest  $N_2O$  emissions (up to  $170 \mu g m^{-2} h^{-1}$ ) from two sites in the vicinity of

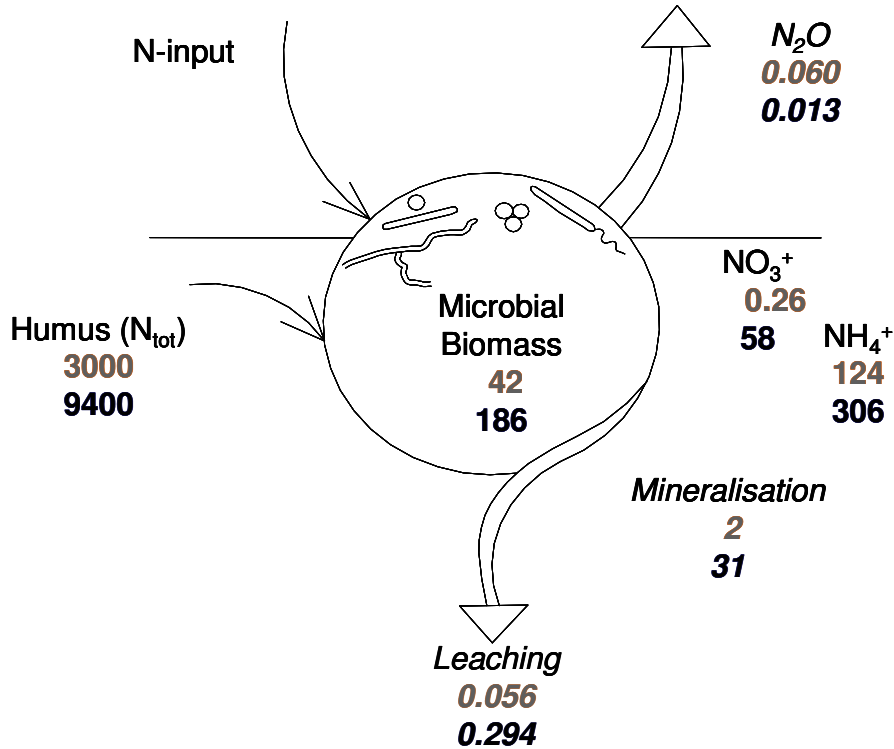


Figure 3: Minimum and maximum N-turnover rates from 12 natural forests: Acidic beech forest Saubrunn (minimum values grey) and spruce-fir-beech forest Rothwald (maximum values black). Pools are depicted in standard script ( $\mu\text{g g}^{-1}$ ), fluxes in italics ( $\mu\text{g g}^{-1} \text{d}^{-1}$ ); data from Hackl et al. (2004a).

Vienna; these high emissions related more to the high rates of N deposition ( $35 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) rather than to the species composition of the forests (Zechmeister-Boltenstern et al., 2002). Beside these two N-affected forests, the floodplain forests showed the highest emissions of  $N_2O$  ( $60 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ ). Soil moisture and nitrate concentrations were the main drivers for  $N_2O$  emission within sites (Hackl et al., 2004a).

Atmospheric methane is typically degraded (oxidation) in upland forest soils, and emitted from forests on peatland or swamps (Ambus and Christensen, 1995). Deciduous forests may take up more methane than conifer forests (Butterbach-Bahl and Papen, 2002). A comparison of 16 sites showed highest methane uptake (oxidation) rates in floodplain forests with high microbial biomass (Zechmeister-Boltenstern and Nikodim, 1999). Methane uptake in forest soils was reduced by increasing inputs of nitrogen and  $CO_2$ -concentrations (Rigler and Zechmeister-Boltenstern, 1999). Methane uptake rates in the natural forests ranged between  $3$  and  $30 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$ . Soil microbiological contribution to the global sink strength is about 6% for  $CH_4$  (Crutzen, 1991). However, methane uptake rates had little effect on the net greenhouse gas exchange of our investigated forests. From Table 2 it can be

Table 2: N<sub>2</sub>O-emissions, CH<sub>4</sub>-uptake (negative values), CO<sub>2</sub>-emissions, and net greenhouse gas exchange calculated as CO<sub>2</sub>-equivalents according to the Third Assessment Report (IPCC, 2001)

Site	N <sub>2</sub> O	CH <sub>4</sub>	CO <sub>2</sub>	Greenhouse gas exchange
	μg CO <sub>2</sub> -E.m <sup>-2</sup> .h <sup>-1</sup>			
JE	78891	-54	66159	144995
K	18392	-26	61558	79923
JB	38246	-71	57730	95905
KI	10374	-95	35643	45922
D	1333	-37	46133	47428
S	685	-101	56984	57567
R	1980	-100	67513	69393
N	14239	-35	50570	64774
M	14000	-181	18180	31999
B	2121	-27	29072	31166
St	484	-16	24750	25218
Me	511	-61	11834	12284

seen that greenhouse gas exchange of forest soils was mainly determined by N<sub>2</sub>O and CO<sub>2</sub> release. The lowest net greenhouse gas release occurred from floodplain and pine forests. These results are potential turnover rates as they result from laboratory incubation experiments. By investigating 30 individual cores for each measurement we may have covered some of the spatial variability, but temporal variability remained large and could not be accounted for (see Butterbach-Bahl and Kiese, this volume).

## Decomposition and C storage

Ecophysiological quotients allow inferences about humus quality and decomposition processes, as well as the physiological state of the soil microflora. In Figure 4A the microbial quotients (C<sub>mic</sub>:C<sub>org</sub>) of the sampling sites are compared. The concept of the microbial quotient as an indicator of successional stage was introduced by Insam and Domsch (1988) and reviewed by Wardle et al. (1995). Here, the microbial quotient is an indicator for substrate quality on top of successional stage. A high microbial quotient means that the soil organic matter can sustain a large microbial community whereas a low microbial quotient would mean that the organic carbon is less palatable for the microbiota. Moreover, it is an indicator of carbon accumulation (Insam, 1988). The floodplain forests had a significantly higher microbial quotient than most other forest types. Floodplain forests, which are still affected by regular floods, may also be seen as forest types of an early successional stage, as compared to forests on higher terraces (Kaye et al.,

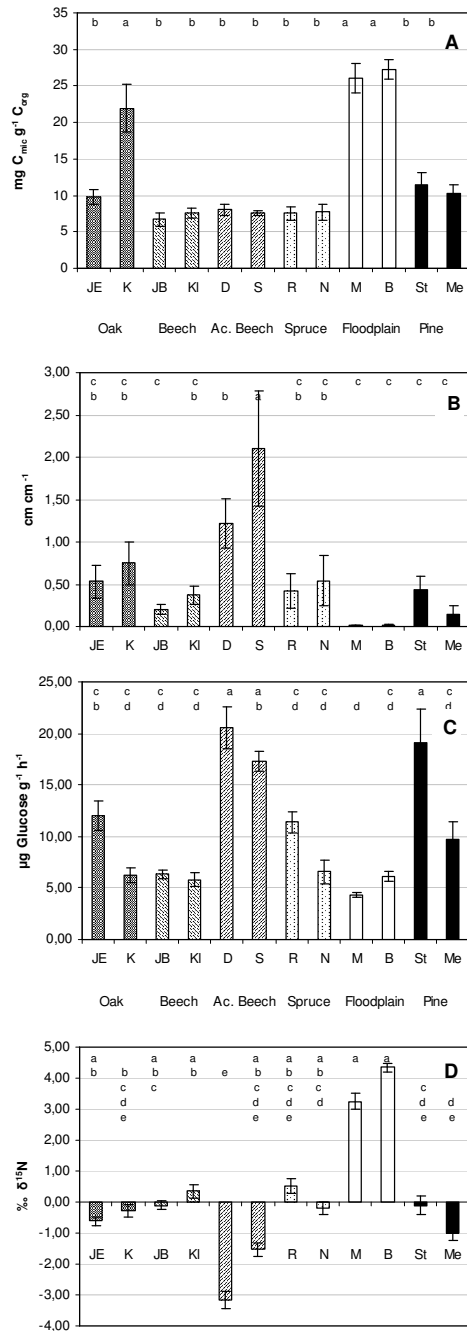


Figure 4: Ecophysiological Parameters providing information on humus quality and decomposition. A: Metabolic quotient B: Thickness forest floor/organic mineral horizon C: Xylanase activity D: δ<sup>15</sup>N of total soil nitrogen.

2003). However this does not necessarily imply that these soils are accumulating organic matter. The high microbial quotient in our floodplain soils occurred with typical microflora of zymogenous or r-strategic organisms, which are able to quickly exploit easily decomposable carbon substrates (Hackl, 2004c; Killham, 1994; Paul and Clark, 1996). Fresh litter is decomposed immediately in the floodplain forests, which have a large fraction of bacteria (Figure 4B; Figure 5). In contrast, the acidic beech forests and the pine forests have a more recalcitrant carbon substrate sustaining a comparably small mass of microorganisms which are known as autochthonous or K-strategists. These microorganisms grow slowly, can survive for a long time under starving conditions and are able to break down recalcitrant substrate. The microbial community in the pine forest soils had a high fraction of fungi and actinomycetes (Figure 5; Hackl et al., 2004c). A high fungal to bacterial ratio in pine forests has also been measured in boreal ecosystems (Šantrůčková et al., 2003). A further indication for recalcitrant C substrate is the high xylanase activity in the pine soils (Figure 4C). Xylan is a component of plant cell walls, it is slowly decomposed and requires specialized microorganisms, such as basidiomycete fungi, for breakdown (Devlin and Witham, 1983; Paul and Clark, 1996).

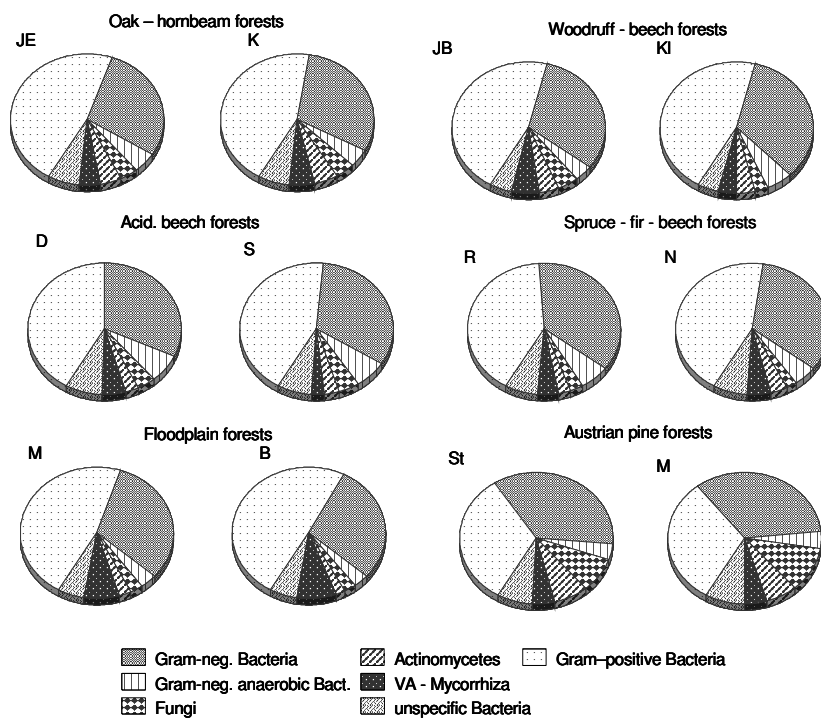


Figure 5: Microbial community composition of 12 natural forest sites according to PLFA analysis.

The metabolic quotient ( $q\text{CO}_2 = \text{CO}_2$  evolved  $\text{g}^{-1}$  microbial biomass) is an indicator of the respiration level of microbes (Insam, 1996). A low metabolic quotient means that the microbial biomass is in a “sleepy state” respiring at a low level. This behaviour is also said to be “efficient” in contrast to a high metabolic quotient which has been observed as a stress phenomenon (Klose et al, 2003; Šantrůčková and Straškraba, 1991). All natural forest soils had a low metabolic quotient of 2-4 except for the pine forests, which reached 18-19. Soils of the pine forests are shallow and dry. Soil moisture was usually at least 10% less than in the other forest soils. On top of a stress phenomenon a high metabolic quotient may also indicate a different microbial community (Šantrůčková and Straškraba, 1991). Indeed, the pine forests contained a high abundance of actinomycetes, which are known for their high respiration activity and occur in warm and dry soils (Figure 5; Hackl et al., 2004c).

Another indication for organic matter accumulation rather than degradation is the ratio of forest floor thickness relative to organic mineral soil (Figure 4B; Nestroy et al., 2000). A thick litter layer was accumulating in the acidic beech forests, whereas all litter was comminuted (and perhaps decomposed) in the floodplain forests each year. This can be explained by the composition of tree species like ash (*Fraxinus excelsior* L.), willow (*Salix alba* L.) and poplar (*Populus alba* L.) as well as the nitrophilous ground vegetation and the likely differences in the forest soil communities. Litter from these plants is easily decomposed (Kaye et al., 2003; Prescott et al., 2000). We speculate that these floodplain forests do not accumulate soil carbon and nitrogen in the organic mineral soil. These forests can be seen as transient ecosystems, which would only reach a higher successional stage if they were cut off from riverine water. In this case different tree species would settle in and the soils might start to accumulate organic matter. Carbon accumulation might also happen in deeper horizons via deep roots, which have been shown to reach down to 2.9 m in floodplain forests (Kutschera and Lichtenegger, 2002).

The ideas described above are consistent with  $\delta^{15}\text{N}$  natural abundance patterns (Figure 4D). Low  $\delta^{15}\text{N}$  values in soil humus indicate the presence of undecomposed plant material whereas a high  $\delta^{15}\text{N}$  typically indicate old, highly processed humus. Values of  $\delta^{15}\text{N}$  typically increase with depth in soil (Haberbauer et al., 2002; Heil et al., 2000), as a long-term outcome of microbial discrimination and losses of  $^{14}\text{N}$ . Microbial enzymes tend to process the lighter  $^{14}\text{N}$ , leading to higher losses of this isotope ( $\text{NO}$ ,  $\text{NO}_2$ ,  $\text{N}_2\text{O}$  gases, or nitrate leaching) (Högberg, 1997; Koba et al., 1998). Our measurements showed highest  $\delta^{15}\text{N}$  values in floodplain forests (Fig 4D), indicating higher rates of N loss. The acidic beech forests had negative  $\delta^{15}\text{N}$ , indicating more N derived from recent plant materials. These ecophysiological soil measurements are consistent with the acidic beech forest soils accumulating C and N, while this is not evident in the organic mineral soil from the floodplain forests.

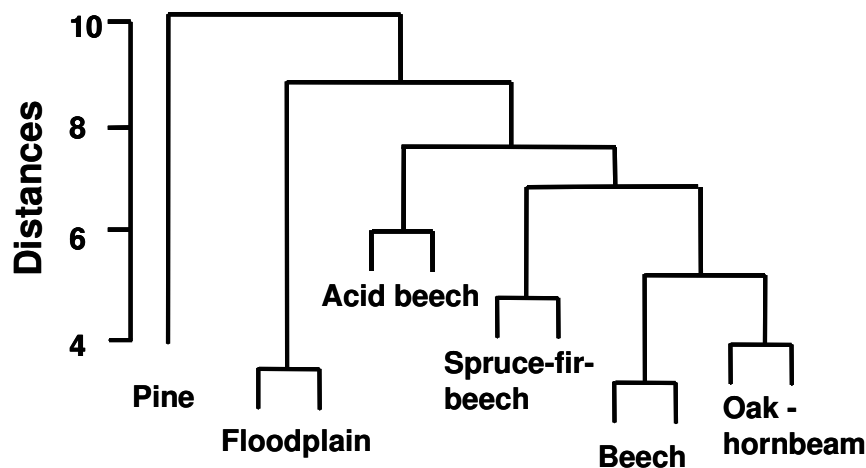
## Biodiversity

All the natural forests showed great diversity of soil organisms, microbes, as well as micro-, meso- and macrofauna (Drapela, 2003; Foissner et al., 2004; Waitzbauer and Zabransky, 2004; Zechmeister-Boltenstern, 2003). More than one thousand species of soil organisms have been identified, including 35 new species (DIANA project: <http://bfw.ac.at/300/2197.html>). These were protozoa (Foissner et al., 2004), as well as one springtail (Pomorski et al., 2003) and one spider (Milasowszky personal communication). Earthworms occurred only in the floodplain forests, where the annual litterfall was comminuted into the mineral soil each year. Oribatid mites were most abundant in acidic beech forest soils (Laibl and Bruckner, personal communication). The floodplain forests had very high species richness of many soil taxa, perhaps resulting from the recurring floods. Many rare and new species were found in pine forests. The spruce-fir-beech forests contained carabid beetles and spiders which are specialized on old-growth forests. This high biodiversity in all the natural forests may not be greater than that of managed forests. For example, spider diversity was significantly lower in the natural forests than in 89 managed forests in Central Europe (Milasowszky personal communication). In managed forests many common and ubiquitous spiders are found due to edge effects and disturbances. The species found in natural forests were specialized forest spiders, which may only survive under the conditions provided in protected areas.

Microbes contribute the largest fraction to soil biomass. Microbial biodiversity and community composition can be determined by PLFAs (Phospholipid fatty acids) analysis. Cluster analysis groups forests according to similarity in the overall PLFA profiles (Figure 6). The PLFA profile in the pine forests differed the most from the other forest types, and the floodplain forests also showed a distinct microbial community composition. The microbiota of oak-hornbeam forests were similar to those of the beech forests, whereas the spruce-fir-beech forests and acidic beech forests were less similar to each other.

Pine forests were rich in actinomycetes and the fungal biomass (Figure 5), perhaps resulting from the recalcitrant pine needle litter which fungi can utilize better than other microorganisms. An investigation of 13 forests across Europe revealed a close similarity of microbial communities in mediterranean pine forests (*Pinus pinaster*, Italy) to those in boreal pine forests (*Pinus sylvestris*, Finland) (Zechmeister-Boltenstern et al., 2004). In spite of the climatic differences pine needles supported similar functional groups of microorganisms which distinguished them from other forest types.

*Figure 6:* Dendrogram of individual sampling sites according to microbial communities as determined by PLFA analysis (Cluster analysis in S-Plus, average linkage procedure).



The floodplain forests contained a large portion of gram positive bacteria, as well as arbuscular mycorrhizal fungi. Bacteria are known to dominate at neutral pH (Paul and Clark, 1996). Arbuscular mycorrhizae occur in symbiosis with herbal vegetation, which is abundant in floodplain forests, as well as in symbiosis with tree species such as ash and poplar. The total amount of PLFAs correlated well with microbial biomass determined by chloroform fumigation extraction, indicating both methods provide the similar estimates of microbial pool sizes. The measurements of spring and autumn samples revealed similar results, indicating that the microbial community had not changed significantly between seasons (Hackl et al., 2004c).

Soil DNA was extracted for a closer view on bacterial populations; 16S rRNA genes were amplified and subjected to terminal restriction fragment length polymorphism analysis. Clone libraries were created and taxonomic assignments were made by comparison with existing databases (Hackl et al., 2004b). The bacterial communities in pine forests were compositionally distinct from those in oak and spruce-fir-beech forests, as we found for the overall microflora. High -G+C gram positive bacteria (mainly belonging to the *Actinobacteria* group) prevailed in the pine forest. High -G+C gram positives were also most prevalent in 16S rRNA clone libraries from mineral forest soil samples in British Columbia, where lodgepole pine (*Pinus contorta* Douglas) was a major tree species (Axelrood et al., 2002). In oak and spruce-fir-beech forests, representatives of the *Holophaga-Acidobacterium* group were most abundant. Only a few cultivated representatives of the *Holophaga-Acidobacterium* group exist, and these bacteria have never been detected by classical methods. Only recently and after the introduction of molecular methods have they been acknowledged as an important bacterial group. Among forest soils, those under pinyon pine-juniper woodlands have been reported to contain acidobacteria in highest numbers (Dunbar et al., 1999; Hackl et al., 2004b).

## CONCLUSIONS

Our investigations showed an influence of forest type on the composition and function of the soil microbial communities. Abiotic factors such as bedrock, moisture and nitrogen deposition were also important in nutrient turnover rates and the emission of greenhouse gases. Ecophysiological quotients could reveal information about humus decomposition and carbon storage, which was not evident from primary data.

When reviewing tree-soil interactions in the light of global climate change, two viewpoints have to be considered. First, how do different forest types affect global climate change? Should management schemes aim to maximize wood production or to conserve the soil carbon? In the first case fast growing tree species with easily decomposing litter and a narrow C:N ratio have to be favoured. This could be species of floodplain forests, such as poplar. Poplar plantations are already used as so called “Kyoto forests” planted for maximum C sequestration in the tree biomass (Leip et al., 2003). In the second case tree species with high lignin concentrations in the litter, and slow microbial decomposition rates (Lovett et al., 2004; Thomas and Prescott, 2000) may maximise carbon conservation. Drainage of forests should then be avoided especially on highly organic soils (Roulet, 2000). In the long term, C sequestration and storage in old-growth forests may be considered more important than short time carbon turnover. This potential must not be underestimated, for example it has been calculated that Siberian forests reassimilate 90% of European fossil fuel emissions (Schimel et al., 2001).

Second, how will global climate change affect forests? Forest types will clearly react differently to changes in climate, and we can offer some speculation in the absence of strong experimentation. Drought probably hampers decomposition in some forests (such as Austrian pine forests), and increased precipitation might increase decomposition. Decomposition in other forests (such as the acidic beech forests) may or may not be limited by low pH, with less response to changes in precipitation. A rise in temperature is most likely to affect soils with nutrient rich litter and organic mineral soils, as found in spruce-fir-beech forests in limestone Alps. These forests have a short vegetation period and longer growing seasons might therefore enhance decomposition rates and losses of soil carbon. The pattern of climate change would also be important, with different responses to slow or rapid changes.

We recommend that afforestation programs should take into account the potential natural vegetation of a site (Bohn et al., 2003; Mayer, 1984; Ricotta et al., 2002). Many people would assume that indigenous tree species will be supported by the native soil community, finding compatible symbiotic partners (including mycorrhizal fungi). Hence their connection to the soil community could be considered as tightly woven, where the fitness of the tree is enhanced by its long-term-effect on soils. This view is a slight adaptation of

the concept by Binkley and Giardina (1998), who considered tree-microbe interactions more frayed. In addition, indigenous trees are adapted to prevalent climate and soil conditions and may therefore be resilient to various stress effects (Grabherr et al., 1995). Our study suggests that natural forest soils have relatively closed nutrient cycles with small losses of nitrate and nitrous oxide. They may be considered as beneficial for the environment and the conservation of site-specific biodiversity

## ACKNOWLEDGEMENTS

This work was financially supported by the Federal Ministry for Agriculture and Forestry, Environment and Water management. We thank R. Jandl for helpful comments on the manuscript and the Kitzler sisters for editing.

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