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THE RHIZOSPHERE EFFECTS OF *FAGUS SYLVATICA* L. AND *FRAXINUS EXCELSIOR* L. SAPLINGS ON GREENHOUSE GAS FLUXES BETWEEN SOIL AND ATMOSPHERE

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Auch das kleinste Ding hat seine Wurzel in der Unendlichkeit, ist also nicht völlig zu ergründen.

nach

Oscar Wilde

SUMMARY

Tree species influence the soil through stemflow and throughfall water, leaf litter and the root system. Little is known about the effects of tree roots on the C and N dynamics of the soil and the gas exchange with the atmosphere. In the present study, the effects of European bech (*Fagus sylvatica* L.) and Common ash (*Fraxinus excelsior* L.) saplings, as important European broad-leaved tree species, on C and N fluxes in the soil of a species-rich temperate forest were investigated under constant climatic conditions. The main objective was to identify root-induced changes in the greenhouse gas fluxes of CO_2, CH_4, and N_2O between soil and atmosphere. A stepwise experimental approach was used to extend the knowledge about rhizosphere effects on soil biogeochemistry. In the first step, the effects of simple C and N alteration by KNO_3 (equivalent to 200 kg N ha^{-1} yr^{-1}) and glucose addition (equivalent to 9419 kg C kg ha^{-1} yr^{-1}) on the fluxes of CO_2, CH_4, and N_2O were investigated for a basic understanding of the C and N dynamics in the incubated forest soil (Chapters 2 and 3). In the next step, the changes due to C and N alteration were compared with the putatively complex effects of ash roots on CO_2 and N_2O emissions in soil columns (Chapter 4). Finally, species-specific effects of the roots of beech and ash saplings on the C and N cycling of the soil were analysed in soil columns and novel double-split-root rhizotrons (Chapters 4, 5, and 6).

The experimental investigation of the effects of NO_3^- and glucose addition on the greenhouse gas exchange (Chapter 2) revealed a large reduction in net CH_4 uptake due to increased N availability and saturating doses of C (reductions up to 86% and 83%, respectively). Moreover, addition of NO_3^- and glucose increased the N_2O emissions by factors of 8 and 39, respectively, whereas the CO_2 efflux remained constant after N addition and increased dramatically up to 11-fold after C addition (Chapter 3). A synergistic effect of C and N addition on all three investigated gas fluxes could be shown. The results of the simple C and N addition experiments suggest that the effect of the large C addition on all three investigated greenhouse gases, including the measured N emissions, was larger than the effect of elevated N availability, which might be important under a variable climate.

The comparison of the effects of N addition and the presence of ash roots on CO_2 and N_2O emissions showed that the ash roots greatly reduced the N_2O emissions by

up to 98%, whereas N addition increased the N_2O emissions just by 54% (Chapter 4). These results indicate that the effect of ash saplings on N_2O might not be exclusively explained by the N uptake of the roots, and that plant species effects of the rhizosphere changes should achieve a higher attention in future studies on the greenhouse gas balance of forest soils.

As in the soil columns, the rhizotron experiment showed a large reduction of N_2O emissions by ash roots (Chapter 5). In contrast, the reduction of N_2O release in presence of beech saplings was only slight or not visible in the rhizotrons and the soil columns (Chapters 4 and 5). The CO_2 emissions from soil planted with ash tended to be higher than, or were similar to, the emissions from soil planted with beech (Chapters 4 and 5). Due to the higher relative contribution of root respiration to total soil respiration in ash rhizotrons (35.5 ± 8.5 vs. 9.0 ± 2.7 %, Chapter 5), we assume that a higher activity of saprotrophic fungi and a larger microbial-specific respiration was responsible for the similar CO_2 effluxes from soil under beech and ash (Chapter 6). In the rhizotron approach, the CH_4 uptake was significantly increased under ash compared to the control soil (Chapter 5), while beech saplings did not significantly affect the CH_4 uptake. In contrast to the observed changes in greenhouse gas fluxes, the C and N stocks of soil under beech and ash were only slightly different. In conclusion, the gas fluxes from the soil to the atmosphere can be used as sensitive indicators of even small changes in the biogeochemical processes of forests.

Despite the higher CO_2 efflux from soil under ash, the greenhouse gas balance calculated as the sum of CO_2, CH_4, and N_2O fluxes tended to be more favourable for soil under ash than for soil under beech saplings in all experiments, which indicates a mitigating influence of European ash on the greenhouse gas balance of temperate forest soils. Further field and laboratory research on the relation between root systems and greenhouse gas fluxes from the soil are needed for realistic predictions of the future greenhouse gas balance under changing climatic conditions.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

a.s.l.	above sea level
AMO	ammonium monooxygenase
BAS	basal respiration
CEC	cation exchange capacity
CH_4	methane
CO_2-eq	CO_2 equivalents
C_{mic}	microbial biomass
C_{org}	organic carbon
DIC	dissolved inorganic carbon
DNRA	dissimilatory nitrate reduction to ammonium
DOC	dissolved organic carbon
dw	dry weight
GC	gas chromatography
GHG	greenhouse gas
N_2O	dinitrogen oxide
N_{total}	total nitrogen
PLFA	phospholipid fatty acid
pMMO	particulate methane monooxygenase
qO_2	microbial specific respiration (BAS/C_{mic})
R_a	autotrophic respiration
R_h	heterotrophic respiration
sMMO	soluble methane monooxygenase
SOM	soil organic matter
WFPS	water-filled pore space

GENERAL INTRODUCTION

1.1 GREENHOUSE GASES AND THE SOURCE/SINK PROCESSES OF C AND N CYCLING IN SOIL

It is unequivocal that the global average surface temperature of the earth has arisen since the beginning of industrialisation about 250 years ago (IPCC, 2007). In first line, the recent climate warming of the earth is a result of increased anthropogenic greenhouse gas emissions (IPCC, 2007; Montzka et al., 2011). The four most influential atmospheric greenhouse gases are water vapour (H_2O), carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O; Forster et al., 2007). In the present study we focus on the greenhouse gases CO_2, CH_4 and N_2O representing major compounds of the global carbon and nitrogen cycles. The study addresses functions of forest soils as sinks and sources for these gases under different abiotic and biotic impacts. The outline in the paragraphs 1.1.1 – 1.1.3 describes the global role of soil for the atmospheric concentrations of the greenhouse gases CO_2, CH_4 and N_2O and copes with the dominant uptake and release processes including the soil.

1.1.1 CO_2

CO_2 as an important component of the global C cycle is the most prominent human-induced greenhouse gas. The recent global radiative forcing of CO_2 is 1.66 W m^{-2} (Forster et al., 2007). Since industrialisation started around 1750, the concentration of CO_2 has increased by 36% from 278 to 379 ppm in 2005 (Forster et al., 2007). Half of this concentration increase in the past three decades, mainly due to anthropogenic CO_2 emissions from the combustion of fossil fuels, gas flaring, cement production, land use changes such as deforestation and biomass burning (Forster et al., 2007).

The high anthropogenic CO_2 emissions of about 8 Gt C yr^{-1} were partially compensated by natural net CO_2 sinks which incorporate about 3 Gt C yr^{-1} (Forster et al., 2007). Carbon (C) assimilation by the marine and terrestrial ecosystems driven by photosynthesis of phytoplankton and terrestrial plants are the dominant processes of CO_2 consumption from the atmosphere. Recently, the net sink strength of terrestrial ecosystems for CO_2 has been intensively discussed (Seneviratne, 2003; Reay et al., 2008; Cuntz, 2011; Welp et al., 2011). After the oceans (38 000 x 10^{15} g C), soils globally are the second largest C pool (1 500 x 10^{15} g C) followed by

the atmosphere (750 x 10^{15} g C) and plant biomass (560 x 10^{15} g C); soils thus have a high potential to store CO_2 (W.H. Schlesinger & Andrews, 2000).

PROCESSES OF CO_2 RELEASE AND UPTAKE IN SOILS

With the decomposition of plant necromass by the decomposer fauna, fungi and microbes, soils house the C assimilated by plants. The C is incorporated in the different fractions of soil organic matter (SOM) such as plant and animal residues at various stages of decomposition (dead SOM), the entity of living soil organisms, i.e. the edaphon (living SOM), and other biogenic substances produced by soil organisms.

In upland (aerobic) soils, the oxidation of SOM is linked to the production of CO_2 (heterotrophic respiration, Fig. 1.1). Additionally, CO_2 is released in large amounts through the respiration of plant roots and their rhizosphere (autotrophic respiration; Kuzyakov & Larionova, 2005). The photosynthesis and autotrophic respiration of algae and chemolithotrophs is of minor importance in most soils (Kuzyakov & Larionova, 2005; Horwath, 2007). In a meta-analysis Subke et al. (2006)

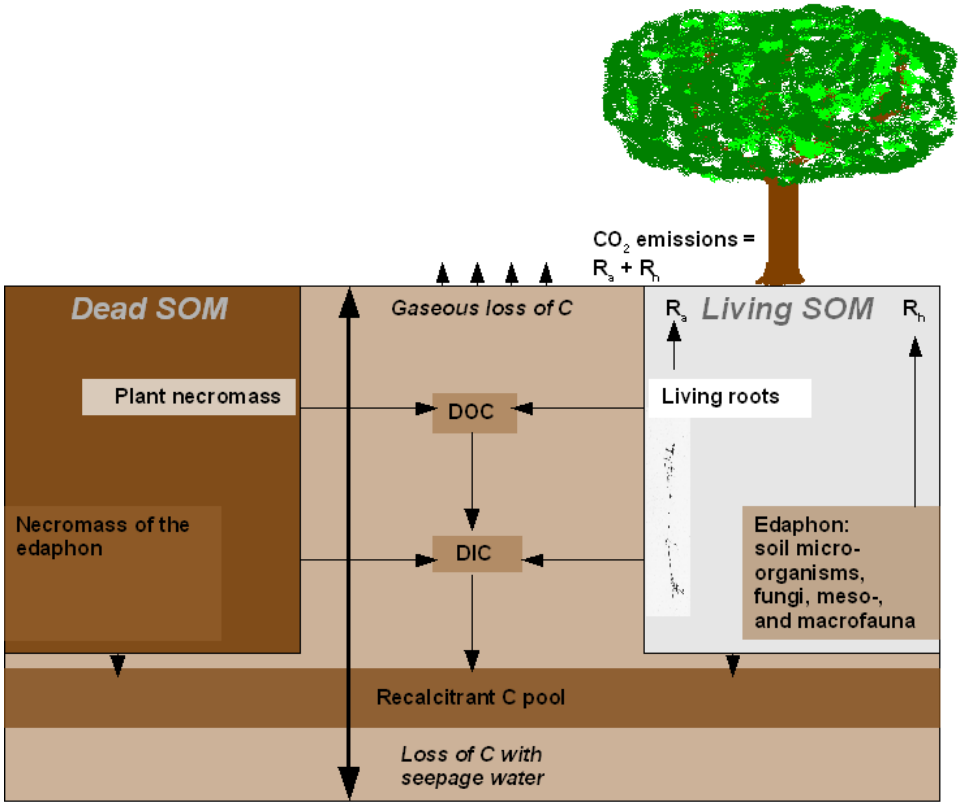

Fig. 1.1 Carbon pools and fluxes in the soil. R_a = autotrophic respiration, R_h = heterotrophic respiration, SOM = soil organic matter, DOC = dissolved organic carbon, DIC = dissolved inorganic carbon.

demonstrated that in different ecosystems the heterotrophic respiration may contribute between 0.03% and 99% to the total soil respiration. To a smaller extent, CO_2 may be lost from an ecosystem by leaching of C containing compounds such as dissolved organic carbon (DOC) or dissolved inorganic carbon (DIC) through rivers or by important singular events such as harvests or fire (Schlesinger & Andrews, 2000; Schulze et al., 2009). In general, the net CO_2 balance of ecosystems is controlled by the ratio of CO_2 assimilation by plants and the emissions of CO_2 by auto- and heterotrophic respiration from the soil.

1.1.2 CH_4

Methane is currently the most abundant anthropogenic non-CO_2 greenhouse gas in the atmosphere (Montzka et al., 2011). Its concentration increased since the start of industrialisation around 1750 from 715 ppb to 1774 ppb in 2005 (Forster et al., 2007). After CO_2, CH_4 has the second-largest human-caused radiative forcing of 0.48 W m^{-2} (Forster et al., 2007). The lifetime estimate of CH_4 is 12 years, and hence its global warming potential for the 100-yr horizon is 25 times that of CO_2 (Forster et al., 2007). In relation to natural sources of CH_4, which are estimated at about 200 Tg yr^{-1}, human activities additionally lead to a release of about 350 Tg yr^{-1} (Denman et al., 2007). The main natural sources of CH_4 to the atmosphere are wetlands (Denman et al., 2007). The most important anthropogenic CH_4 sources are energy production from coal and natural gas, waste disposal in landfills, raising of cattle breeding, rice agriculture and biomass burning are human-made CH_4 sources (Denman et al., 2007).

With 511 Tg yr^{-1}, the main sink for CH_4 is the chemical oxidation in the troposphere by hydroxyl radicals ($OH^{\bullet}$; Crutzen, 1991; Denman et al., 2007). Beside, the losses of CH_4 to the stratosphere and the biological oxidation in aerobic soils are smaller, but nevertheless significant sinks for atmospheric CH_4 (Smith et al., 2000; Le Mer & Roger, 2001; Denman et al., 2007). The contribution to the global CH_4 sink is calculated to 40 Tg yr^{-1} for the stratospheric loss and to 22.4 – 30 Tg yr^{-1} for the oxidation in soils (Denman et al., 2007; Dutaur & Verchot, 2007). The CH_4 uptake of soils in the temperate zone accounts for 30 – 50% (10.4 Tg CH_4 yr^{-1}) of this global soil-driven sink (Dutaur & Verchot, 2007).

PROCESSES OF CH_4 RELEASE AND UPTAKE IN SOILS

The aerobic CH_4 oxidation in soils is carried out by methantrophic bacteria and nitrifiers. The aerobic methanotrophs are a group of methylotrophic bacteria, which use CH_4 and other mono-carbon-compounds as their only energy and C source (Trotsenko & Murrell, 2008; Dedysh & Dunfield, 2011). They have enzymes (particulate and soluble methane monooxygenase, pMMO and sMMO, respectively), which catalyse the oxidation of CH_4 to methanol (Dedysh & Dunfield, 2011). In wetland soils, numerous methanotrophic organisms belonging to the bacteria are well investigated, which exhibit enzymes with a low affinity but a high capacity to oxidise CH_4. In contrast, most CH_4 is consumed by poorly studied methanotrophic bacteria, which exhibit enzymes with a high affinity to CH_4 in upland soils (Bodelier, 2011a). Nitrifiers may use CH_4 in aerobic soils due to the similar structure with the NH_4^+ oxidising enzyme ammonium monooxygenase (AMO) and a close evolutionary relation of AMO and pMMO (Bédard & Knowles, 1989; Holmes et al., 1995).

Methane is produced by methanogenic organisms under strictly anaerobic conditions, when redox potentials are low (Smith et al., 2003), e.g. in soils of mires, swamps, flooded rice fields and wet forests. The methanogens, belonging to the domain *Archaea*, breakdown organic compounds and use them as C and energy source (Le Mer & Roger, 2001). In most upland soils, the CH_4 consumption processes of methane-oxidising bacteria exceed the production of CH_4 by methanogens.

1.1.3 N_2O

N_2O is the third most important anthropogenic greenhouse gas with a radiative forcing of 0.16 W m^{-2} (Forster et al., 2007). Its atmospheric concentration has increased from 270 ppb in pre-industrial times by 19% to 319 ppb in 2005 (Forster et al., 2007). The greenhouse gas with a long lifetime of 114 years has a 298 times higher global warming potential than CO_2 for the 100-year horizon (Forster et al., 2007). Human-driven N_2O emissions by agricultural practices e.g. the use of inorganic N fertilisers and cultivation of nitrogen-fixing crops (Denman et al., 2007) and industrial processes such as combustion of fossil fuels (Galloway et al., 2008), resulted in a global source strength of 4.1 – 8.1 Tg N yr^{-1} (Denman et al., 2007). Soils below natural vegetation represent the main natural global source of atmospheric N_2O (with 6.6 Tg N yr^{-1}; Denman et al., 2007).

PROCESSES OF N_2O RELEASE AND UPTAKE IN SOILS

The net N_2O uptake by soils has only recently been reported by Chapuis-Lardy et al. (2007). The suggested main processes of N_2O reduction in soils are denitrification of N_2O to N_2 and nitrifier denitrification (processes are described below). However, the uptake rate is rather small compared to the high N_2O effluxes from soils, and thus, plays a minor role for the N_2O exchange with the atmosphere (Chapuis-Lardy et al., 2007).

Soils release N_2O which is produced predominantly biologically in soil during denitrification, nitrification, dissimilatory nitrate reduction to ammonium (DNRA, also referred to as nitrate ammonification) and nitrifier denitrification (Fig. 1.2; Baggs & Philippot, 2010; Baggs, 2011). These processes occur simultaneously within microsites of the same soil (Robertson & Tiedje, 1987; Baggs, 2008). Autotrophic nitrification and denitrification are the two main processes leading to the production of N_2O in upland soils (Bateman & Baggs, 2005). The other processes like DNRA, heterotrophic nitrification by fungi and anaerobic oxidation of ammonium were recently highly discussed, but their contribution to the N_2O emissions is not sufficiently understood yet (Wrage et al., 2001; Wolf & Brumme, 2003; Dalsgaard et al., 2003; Ambus et al., 2006; Morley & Baggs, 2010).

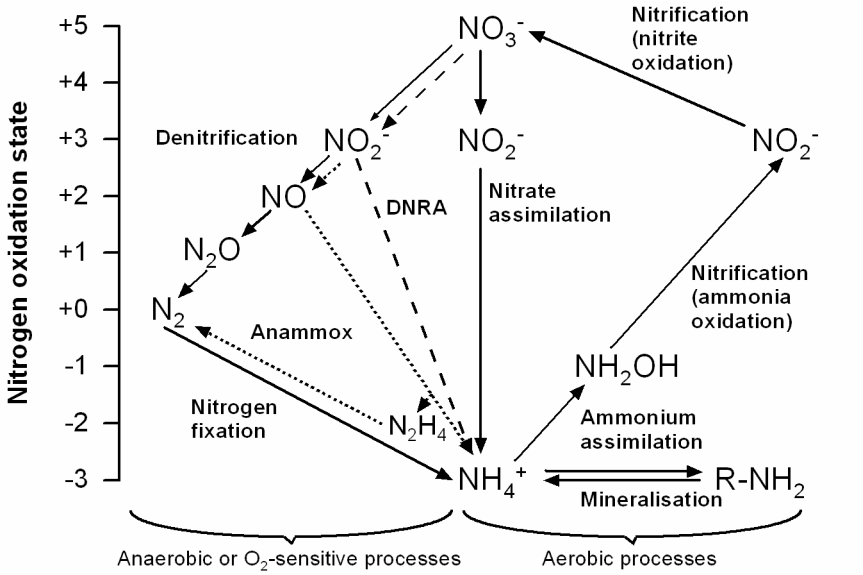

Fig. 1.2. The N dynamics in soil according to the oxidation states. The main oxidative or reductive pathways are indicated by solid arrows. Anaerobic ammonia oxidation (anammox) is indicated by dotted arrows and dissimilatory nitrate reduction to ammonium (DNRA) by dashed arrows. In the left the oxygen-sensitive reactions and anaerobic processes are shown and in the right the aerobic processes are reported; after Cabello et al. (2009).

Denitrification is a process where nitrate (NO_3^-) is reduced to nitrite (NO_2^-), and the gaseous nitric (NO) and nitrous oxide (N_2O) sequentially are reduced and serve as alternative terminal acceptors for electron transport phosphorylation forming N_2 as the final product (Cabello et al., 2009). Under anaerobic conditions, denitrification allows an oxygen-independent respiration of a wide variety of microbial groups of the *Archaea* and *Proteobacteria* (genera *Pseudomonas, Alcaligenes* and to a lesser extent *Bacillus, Agribacterium,* and *Flavibacterium*; Robertson & Groffman, 2007) and even of certain fungi (Coyne, 2008; Cabello et al., 2009). The main controlling factors for denitrification are O_2 concentration, NO_3^- availability in soil and C supply (Firestone & Davidson, 1989). The O_2 concentration is strongly affected by the water-filled pore space (WFPS). Denitrification requires available organic C as energy source to catabolise NO_3^- (Wrage et al., 2001). Beside these main effects for the gaseous loss of N, numerous factors including soil texture, pH and temperature affect the rates and composition of nitrogenous emissions from soil (Voroney & Derry, 2008). Even small changes in one of these factors may alter the N_2O production efficiently, thus, the N_2O fluxes of soil are extremely variable in space and time (Jungkunst et al., 2008).

For a long time, it has been believed that DNRA is a strict anaerobic process in which nitrate is reduced via nitrite to ammonium (Cabello et al., 2009). Just recently, the possible role of dissimilatory nitrate reduction to ammonium (DNRA) under non-strict anaerobic conditions has been realised (Morley & Baggs, 2010). Little is known about the contribution of DNRA to the production of N_2O yet. Results of Morley and Baggs (2010) indicate that a remarkable part of N_2O is produced during DNRA not only under anaerobic conditions. It is suggested that, especially in the rhizosphere, root-derived C flow, high O_2, and nitrate-demands of roots create optimum conditions for the microbial community involved in DNRA (Baggs, 2011).

Nitrification is the biological oxidation of reduced N as ammonia (NH_3) and ammonium to nitrite and nitrate (Norton, 2008; Cabello et al., 2009). The classical process of nitrification involves the sequential oxidation of ammonium to nitrite via hydroxylamine and nitrite to nitrate. It is carried out by *Nitrosomas* and *Nitrobacter* species (Cabello et al., 2009). Nitrifier denitrification is a process of nitrification, where NH_3 is oxidised to nitrite and the subsequent reduction of nitrite to nitric oxide

(NO), nitrous oxide (N_2O) and molecular nitrogen (N_2; Wrage et al., 2001). The involved bacteria belong to the autotrophic nitrifiers (Wrage et al., 2001).

For a deeper understanding and the possible mitigation of the CO_2, CH_4 and N_2O emissions from the soil, it is essential to know more about the production and consumption processes of CO_2, CH_4 and N_2O in different soil types. The knowledge about the regulation of the biogeochemical soil system not only by abiotic, but also by biotic factors should be improved.

1.2 ROLE OF FOREST SOIL FOR THE EUROPEAN GREENHOUSE GAS BALANCE

Forest soils play a key role in the global carbon (C) and nitrogen (N) cycles with a substantial impact on the greenhouse gas balance of the earth (Mosier, 1998; Luyssaert et al., 2010; Duncan C. McKinley et al., 2011). Estimates of the European C balance of the 25 member states of the European Union indicate a strong CO_2 sink of about -274 Tg C yr^{-1}, whereby this high CO_2 sink is dramatically reduced by the emissions of N_2O and CH_4, so that the European greenhouse gas balance equals -29 Tg C yr^{-1} (Schulze et al., 2009). The net greenhouse gas (GHG) balance of European forests was recently estimated at -19 ± 11 g Ceq-CO_2 m^{-2} yr^{-1}, indicating a higher net uptake of GHG by forests than by grasslands, peatlands and croplands, which show net balances between -14 and +44 g Ceq-CO_2 m^{-2} yr^{-1} (Schulze et al., 2010)

Currently, it is estimated that two third (53 g C m^{-2} yr^{-1}) of the net C uptake by European forests are sequestered by the woody biomass increments of the trees, while the remaining third (22 g C m^{-2} yr^{-1}) is fixed in the forest soil (Luyssaert et al., 2010). This total ecosystem net sink for C strongly depends on the CO_2 efflux of the soil, which dominate total ecosystem respiration in forests (Valentini et al., 2000). Heterotrophic respiration of European forest soils was estimated at a mean rate of 368 ± 107 g C m^{-2} yr^{-1}, autotrophic respiration contributes another 507 ± 152 g C m^{-2} yr^{-1} (Luyssaert et al., 2010). In many ecosystem models, it is predicted that climate warming will stimulate microbial decomposition of soil organic matter, and thereby the heterotrophic respiration in the future (Davidson & Janssens, 2006; Friedlingstein et al., 2006). But until now, this enhancement of decomposition due to higher temperatures has been highly disputed. Hence, the consequences of changes in soil respiration for the net C sink of European forests are not totally resolved (Allison et

al., 2010). In different broad-leaved and needle-leaved forests, the relative contribution of heterotrophic and autotrophic soil respiration is estimated to 42 – 84%, and 16 – 58%, respectively (Hanson et al., 2000; Sulzman et al., 2005; Rodeghiero & Cescatti, 2006; Brumme et al., 2009). Generally, the CO_2 emissions of the soils are higher in broad-leaved forests than in needle-leaved forests (Berger et al., 2010; Paré et al., 2011). Vesterdal et al. (2012) measured significant differences among the CO_2 effluxes of soil under different broad-leaved tree species. In a common garden experiment with 30-year old ash, beech, lime, maple, and oak trees the authors found lowest soil respiration under beech, followed by lime, spruce, oak and maple and highest soil respiration under ash.

Regarding the global CH_4 sink, aerobic soils of temperate forests around the globe remarkably contribute with the remarkably high rate of 1.4 – 5.7 Tg CH_4 yr^{-1} to the total consumption of CH_4 by oxidation in soils (22.4 Tg yr^{-1}; Curry, 2007; Dutaur & Verchot, 2007; Ishizuka et al., 2009; Grunwald et al., 2012). The contribution of different forest types such as broad-leaved forests to the global CH_4 balance are not yet estimated. In field and laboratory studies, it was shown that the CH_4 uptake of broad-leaved forest soils is higher than the uptake of needle-leaved forest soils (Borken et al., 2003; Menyailo & Hungate, 2006; Degelmann et al., 2009). In several comparative studies to the CH_4 uptake of beech and spruce forest soil, the CH_4 uptake from the beech sites was higher than that of spruce sites (Butterbach-Bahl & Papen, 2002; Klaus Butterbach-Bahl et al., 2002; Borken & Beese, 2006). However, the comparison of the effects of pedunculate oak and Norway spruce revealed no differences in CH_4 uptake after 15 and 40 years of afforestation (Christiansen & Gundersen, 2011). Guckland et al. (2009) detected no difference in the CH_4 fluxes of the soil among forest patches with varying abundance of beech in a mixed broad-leaved deciduous forest (beech in mixture with ash, lime, hornbeam and maple). Until now, the findings to the differing effects of broad-leaved tree species on CH_4 uptake are scarce.

Beside agricultural soils, upland forest soils are considered as the main natural N_2O sources in Europe (Schulte-Bisping et al., 2003; Kesik et al., 2005). The contribution of European forests to the global N_2O emissions is still not fully understood (Pihlatie et al., 2005). However, it has been recognised that, on a global scale, forests may

have the greatest source potential for future rising N_2O emissions (Reay et al., 2008). The N_2O emissions of broad-leaved forest soils were higher than those from needle-leaved forest soils (Butterbach-Bahl et al., 1997; Butterbach-Bahl & Kiese, 2005; Ambus et al., 2006). In different mixed deciduous sites in National Park Hainich Guckland et al. (2010) showed that the abundance of beech has a high influence on N cycling within the soil what was nevertheless not visible in differing N_2O emissions, possibly due to an efficient retention of N in the soils.

1.3 IMPACTS OF TREE SPECIES ON C AND N CYCLING OF THE SOIL

Beside the known effects of abiotic parameters on GHG fluxes, the influences of biotic factors such as tree species identity and the activity of soil fauna, fungi and the soil microbial community on soil processes, that may affect gas exchange from terrestrial ecosystems with the atmosphere, have received increased attention during the past decades (Binkley & Menyailo, 2005). However, the processes underlying the source-sink relations and the consequences of changing one of these biotic parameters on GHG fluxes are poorly studied (Hanson et al., 2000; Matamala et al., 2003; Paterson et al., 2007; Vargas & Allen, 2008). Recent studies reported significant tree species effects on the CH_4, N_2O and CO_2 fluxes from the soils of European deciduous forests (Borken & Beese, 2006; Degelmann et al., 2009; Vesterdal et al., 2012). The impact of tree species on these gas fluxes have been explained by an alteration of physical and chemical properties of the soil as a consequence of stemflow and throughfall (Hagen-Thorn et al., 2004), leaf litter input (Erickson et al., 2002; Guckland et al., 2009, 2010; van Haren et al., 2010), and root activity (Zechmeister-Boltenstern et al., 2005). In most cases, tree species-related effects on CH_4 uptake or N_2O release have been thought to be caused by the input of leaf litter and its specific properties (Hagen-Thorn et al., 2004; Papen et al., 2005; Chapman et al., 2006; Vesterdal et al., 2012). Although the effects of leaf litter quality and throughfall on soil C and N cycling were frequently investigated (Nordén, 1994; Smith & Bradford, 2003; Knorr et al., 2005; Hobbie et al., 2006; Hansen et al., 2009b), the effects could not be separated from the effect of root activity under field conditions. It is known that the effects of roots on the C and N cycling in the soil are larger than that of most other biotic factors (Brady & Weil, 2002). Until now, the effect of root growth as a factor possibly influencing the GHG

balance of forest soils has received little attention, although it is known that roots actively change the state of the rhizosphere.

1.3.1 The rhizosphere

It is generally accepted that the activity of fine roots (roots < 2 mm in diameter) changes its immediate surrounding by releasing rhizodeposits, water uptake and nutrient uptake (to maintain and increase biomass), decaying root material, respiration (CO_2 production and O_2 consumption) as well as physical changes caused by root growth (Rovira, 1965; Hinsinger et al., 2005; Cheng & Gershenson, 2007). Hence, important biochemical and physical properties such as soil moisture, pH, the redox potential, base saturation, O_2, and CO_2 concentrations, and the labile C and N content in the immediate surrounding of the roots are substantially different from those of the bulk soil (Gregory & Hinsinger, 1999). The term rhizosphere was firstly coined in 1904 and it is defined as the zone surrounding roots, which is influenced by their activity (Brimecombe et al., 2007). The soil biota can be stimulated or inhibited at the rhizoplane (root surface) or in the rhizosphere. The rhizosphere is a highly complex habitat of extreme spatial and temporal heterogeneity, resulting in a patchy inhabitation of numerous soil organisms and forming a specific heterotrophic rhizosphere food web (Uren, 2007). Thus, the investigation of rhizosphere processes is a sophisticate challenge, which is currently under considerable progress by the development of complex new methods for microscale process analyses and molecular techniques (Hinsinger et al., 2005; Jones & Hinsinger, 2008; Brzostek & Finzi, 2011).

Rhizodeposition is a key factor influencing carbon and nitrogen budgets as well as affecting the microbial community in the proximity of roots (Nannipieri et al., 2007). Roots can release protons, oxygen and water (Nannipieri et al., 2007). Beside that, significant quantities of three types of organic compounds are exuded at the root surface, especially at the root apex. First, low-molecular weight organic compounds are released including organic acids, amino acids, and phenolic compounds by root cells. Second, high-molecular weight mucilage is secreted by root-cap cells and epidermal cells near apical zones. And third, cells slough off as the root grows (Brady & Weil, 2002). Rhizodeposition is thought to account for 2 to 30% of total dry-matter production in young plants (Brady & Weil, 2002). Rhizodeposits, in

particular low-molecular weight compounds such as low-molecular organic acids, are chemo-attractant signals to microbes, detoxifiers of aluminium, chelators of poorly soluble mineral nutrients (e.g. P and Fe), and serve as nutrient and energy sources for soil organisms (Dakora & Phillips, 2002). In addition, they may stimulate microbial growth by a higher labile C availability due to priming effects on SOM decomposition (Cheng & Kuzyakov, 2005; Kuzyakov, 2010a), which may accelerate decomposition by up to four times.

Mycorrhizal fungi are involved in the decomposition of organic materials, and thus, improve nutrient acquisition of the host plant but also of other soil organisms (Martin et al., 2007). For all these reasons, it is not surprising, that the rhizosphere is a hotspot of biological activity compared to the bulk soil (Cheng & Gershenson, 2007; Nannipieri et al., 2007).

Regarding the net C gain of soils, the fast C turnover in the rhizosphere, which is sensitive to the increasing atmospheric CO_2 concentrations, rising temperature and additional N inputs to the soil, has to be taken into account (Pregitzer et al., 2007; Kuzyakov, 2010a). Hitherto, the investigation of the rhizosphere focussed predominantly on cereal crops and grass species with particular interest in plant nutrition and fertilisation. Only few studies have been conducted on the rhizosphere of trees (Cheng & Gershenson, 2007; Nannipieri et al., 2007). Due to the high importance of forests for the global C and N budgets, a closer look, especially to the C and N flows in the rhizosphere of trees, is necessary to close the lack of understanding of belowground C and N dynamics in forests (Cheng & Gershenson, 2007). A first step is the experimental investigation of root-induced changes in belowground biogeochemical processes, and their consequences on GHG fluxes under constant climatic and soil conditions.

1.4 STUDY OBJECTIVES AND HYPOTHESES

The present PhD study was conducted as part of an interdisciplinary project: "Biodiversity Manipulation in Rhizosphere and Soil – MicroRhizo" of the Functional Biodiversity Research Cluster of Excellence at Göttingen University. In cooperation with four other PhD students, the rhizosphere of beech and ash was investigated in laboratory experiments under controlled abiotic conditions using homogenised soil

material. For that purpose, novel double-split-root rhizotrons were developed and filled with soil from Hainich National Park, Thuringia, Germany. The interdisciplinary experimental approach aimed at the disentanglement of the influences of diverse soil biota in the rhizosphere of *Fagus sylvatica* L. (European beech) and *Fraxinus excelsior* L. (European ash). The main objective of the present study was to identify the species-specific effects of beech and ash roots (main root characteristics are listed in Table 1.1) on C and N dynamics in the forest soil and the gas exchange between soil and atmosphere. Therefore, a stepwise approach of experimental setups of increasing complexity was followed addressing the dynamics of C and N fluxes in simple soil columns as well as in complex double-split-root rhizotrons.

In the **absence** of roots, the study aimed to consider

I. the effects of labile C and mineral N addition on the CO_2, N_2O and CH_4 gas exchange of the soil in simple soil columns (Chapters 2 and 3). This first step enables a basic understanding of biogeochemical consequences of labile C and N changes without the complicating effects caused by the presence of roots.

In the **presence** of roots, this study focused on

II. the influence of beech and ash saplings in comparison to the effect of mineral N addition together with the resulting complex C and N changes in soil on the CO_2 and N_2O emissions in soil columns (Chapter 4).

III. the multiple paths of influence of beech and ash saplings on soil C and N dynamics and the resulting greenhouse gas exchange in double-split-root rhizotrons. On the one hand, we analysed changes in organic acids in the soil solution as well as O_2 and CO_2 concentrations in the rhizosphere of ash (parts are included in Chapter 5), and on the other hand the effects of beech and ash saplings on the C and N processing associated with leaf litter decomposition in soil were examined (Chapter 6).

Within the Chapters 2 – 6 of this thesis, the following hypotheses were tested:

Chapter 2: (1) high NO_3^- input leads to a relevant reduction in the CH_4 uptake of the forest soil, (2) glucose neither enhances nor inhibits CH_4 uptake when added alone,

but (3) in combination with NO_3^-, it increases the inhibiting effect of NO_3^- on CH_4 uptake.

Chapter 3: (1) even though many forest soils contain large stocks of C, the availability of labile C for N_2O emissions is limited, with the consequence that repeated high N inputs do not result in a corresponding additive increase of the N_2O release rate, and (2) high amounts of labile C in forest soils lead to similar N_2O emission rates as the application of high N amounts, as it was found in studies on non-forest soils (Weier et al., 1993; Bateman & Baggs, 2005). We further hypothesised (3) that the addition of labile C to forest soil affected by chronic N addition must result in very high N_2O emissions exceeding the sum of rates measured in soils treated with N or C sources alone.

Chapter 4: (1) ash saplings reduce the N_2O emissions from the soil significantly due to high root growth and root N uptake rates, which deplete soil N availability, while (2) the emission-reducing effect is smaller in slower growing beech. We further tested the hypothesis (3) that possible emission-reducing effects of the two tree species are small compared to the effect of increased NO_3^- availability on N_2O release.

Chapter 5: (1) the N_2O emissions are higher from soils under ash due to a higher C and N supply in the soil than under beech, (2) the CO_2 efflux from the soil is higher under ash than under beech due to an assumed higher root growth activity, while (3) CH_4 uptake is not different between soil planted with ash or beech saplings, and (4) the interaction of beech and ash roots affects the GHG fluxes in a way that is not simply an additive effect of the fluxes in the monocultures. In order to investigate only root-induced effects on GHG fluxes, we excluded the decomposition of leaf litter as a confounding process in our experimental design.

Chapter 6: (1) beech and ash differentially affect the structure of the microbial community thereby modifying soil processes and plant nutrient capture. Differences in microbial community structure are expected to (2) result in differential decomposition of labeled ash litter and differential mobilization of nutrients from the litter. Further, we expected (3) modifications of the soil microorganisms community and soil processes to be most pronounced in the mixed treatment with both tree

species present due to a complementary effect on soil microorganisms and soil processes.

1.5 STUDY MATERIALS AND EXPERIMENTAL DESIGN

For all experiments we used soil and plant material from the mixed broad-leaved temperate forest in the south-east of Hainich National Park, Thuringia, Germany (51°04' N 10°30' E, about 350 m a.s.l.). This national park represents a species-rich broad-leaved forest of up to 14 co-occurring tree species per hectare. The climate is characterised as sub-atlantic to sub-continental with a mean annual temperature of 7.5 °C and a mean annual precipitation of 590 mm (Deutscher Wetterdienst, 2005). At the sampling site (Lindig), the forest predominantly consists of *Fagus sylvatica* L., *Fraxinus excelsior* L., *Tilia cordata* P. Mill, *Tilia platyphyllos* Scop., *Carpinus betulus* L., *Acer pseudoplatanus* L., and *Acer platanoides* L.

Two species with largely different morphologies, physiologies and phylogenies that are co-occurring in several broad-leaved forest communities of Central Europe and are of moderate to high economic importance for forestry (Ellenberg & Leuschner, 2010) were chosen. The selected tree species, European beech (*Fagus sylvatica* L.) and European ash (*Fraxinus excelsior* L.), represent different families (Fagaceae and Oleaceae) and contrast in their successional statuses (late-successional vs. early-/mid-successional). The species differ with respect to root morphology, type of mycorrhizae, root tip abundances and specific root surface area (Table 1.1).

Table 1.1. Root morphology of beech and ash; after Hölscher et al. (2002) and Meinen et al. (2009). Shown are mean ± 1 SE.

	Mycorrhization	**Branching intensity**	**Specific root tip abundance [number mg^{-1} dw]**	**Specific fine root area [cm^2 g^{-1}]**	**Average root diameter [mm]**
***Fagus sylvatica* L.**	Ectomycorrhized	High	40.2 ± 3.5	394 ± 25	0.38 ± 0.01
***Fraxinus excelsior* L.**	Arbuscular mycorrhized	Low	3.0 ± 0.05	289 ± 10	0.60 ± 0.02

Like the plant material, the soil used in the present study was collected in the Hainich National Park. Although we sampled the soil in a small area, the initial soil physical and chemical properties, such as pH C_{org}, and clay content differ somewhat among the experiments (Table 1.2). An overview of the conducted experiments is given in Table 1.2.

Table 1.2. Summary of the experimental setup, the experimental duration, the climatic conditions, initial physical and chemical soil properties and the initial plant data of the experiments in the present PhD study.

	Chapter 2[1]	Chapter 2[1]	Chapter 3[1]	Chapter 3[1]	Chapter 4	Chapter 4	Chapter 5[2]	Chapter 6[2]
Experimental system	Soil columns	Soil columns	Soil columns	Soil columns	Soil columns	Soil columns	Rhizotrons	Rhizotrons
Name of the sub-experiments	Main experiment	Supplementary experiment	Main experiment	Supplementary experiment	Experiment A	Experiment B		
Target variable(s)	CH_4 uptake of the soil	CH_4 uptake of the soil	N_2O and CO_2 effluxes from the soil	N_2O and CO_2 effluxes from the soil	N_2O and CO_2 effluxes from the soil	N_2O and CO_2 effluxes from the soil	CO_2, CH_4 and N_2O fluxes of the soil	Changes in C and N dynamics of the soil
Determining factors	KNO_3, glucose, KNO_3 x glucose	KNO_3, glucose, K_2SO_4	KNO_3, glucose, KNO_3 x glucose	KNO_3, glucose	KNO_3, ash	Beech, ash, mixture of beech and ash	Beech, ash, mixture of beech and ash	Beech, ash, mixture of beech and ash
Time between establishing the systems and starting the measurements of target variables [d]	62	6	62	6	185	54	151	475
Duration of the experiment [d]	62	20	83	41	28	28	324	475
Duration of daytime [h]	14	14	14	14	14	12	14	14
Light intensity [µmol PPFD m^{-2} s^{-1}]	100	100	100	100	100	200	200	200
Relative air humidity [%]	n. d.[3]	n. d.[3]	n. d.[3]	n. d.[3]	n. d.[3]	80	70	70
Air temperature [°C]	24	n. d.[3]	24	n. d.[3]	25	20	20	20
Soil temperature [°C]	23	n. d.[3]	23	n. d.[3]	23	n. d.[3]	19	19
Dry weight of incubated soil [kg soil system^{-1}]	3.1	3.1	3.1	3.1	3.1	4.7	~15	~15
Soil texture	1.8 % sand, 80.2 % silt, 18.1 % clay	n. d.[3]	1.8 % sand, 80.2 % silt, 18.1 % clay	n. d.[3]	1.8 % sand, 80.2 % silt, 18.1 % clay	2.9% sand, 56.5% silt, 40.6% clay	1.8 % sand, 80.2 % silt, 18.1 % clay	1.8 % sand, 80.2 % silt, 18.1 % clay
Soil water content	40 – 80 % WFPS	14.3 – 33.8 grav.-%[5]	40 – 80 % WFPS	14.3 – 33.8 grav.-%[5]	40 – 80 % WFPS	60 – 76 % WFPS	21 vol.-%[5]	21 vol.-%[5]
Bulk density [g cm^{-3}]	1.05 ± 0.01	n. d.[3]	1.05 ± 0.01	n. d.[3]	1.05 ± 0.01	1.11 ± 0.01	1.04 ± 0.17[4]	1.04 ± 0.17[4]
CEC [µmolc g^{-1} dw]	191.7 ± 11.8	n. d.[3]	191.7 ± 11.8	n. d.[3]	191.7 ± 11.8	n. d.[3]	191.7 ± 11.8	191.7 ± 11.8
Base saturation [%]	22.9 ± 1.3	n. d.[3]	22.9 ± 1.3	n. d.[3]	22.9 ± 1.3	n. d.[3]	22.9 ± 1.3	22.9 ± 1.3
pH (H_2O)	4.58 ± 0.14	4.88 ± 0.02	4.58 ± 0.14	4.88 ± 0.02	5.02 ± 0.14	6.13 ± 0.08[6]	4.56 ± 0.03	4.56 ± 0.03
pH (KCl)	3.82 ± 0.03	n. d.[3]	3.82 ± 0.03	n. d.[3]	4.16 ± 0.08	5.33 ± 0.02	3.95 ± 0.02	3.95 ± 0.02
C/N [g g^{-1}]	10.5 ± 0.2	14.7 ± 0.3	10.5 ± 0.2	14.7 ± 0.3	10.5 ± 0.1	10.5 ± 0.3[6]	11.7 ± 0.14	11.7 ± 0.14
C_{org} [g kg^{-1} dw]	19.8 ± 0.4	29.2 ± 0.5	19.8 ± 0.4	29.2 ± 0.5	19.8 ± 0.4	18.2 ± 0.2[6]	19.2 ± 0.3	19.2 ± 0.3
N_{total} [g kg^{-1} dw]	1.89 ± 0.03	1.99 ± 0.02	1.89 ± 0.03	1.99 ± 0.02	1.88 ± 0.02	1.75 ± 0.04[6]	1.64 ± 0.01	1.64 ± 0.01
N-NO_3 [mg kg^{-1} dw]	6.4 ± 0.3	3.3 ± 0.6	6.4 ± 0.3	3.3 ± 0.6	14.1 ± 3.7	39.0 ± 12.6[6]	6.4 ± 0.3	6.4 ± 0.3
N-NH_4^+ [mg kg^{-1} dw]	7.9 ± 0.3	4.8 ± 0.3	7.9 ± 0.3	4.8 ± 0.3	6.6 ± 1.0	1.5 ± 0.1[6]	7.9 ± 0.3	7.9 ± 0.3
Age of the saplings [yr]	.	.	.	.	5 – 7	3 – 6	3 – 7	3 – 7
Number of buds	.	.	.	.	2 – 4	4 – 13[7]	2 – 5	2 – 5
Shoot height[8] [cm]	.	.	.	.	31.6 ± 2.7	19.4 ± 1.3 / 18.3 ± 0.8	17.9 ± 1.1 / 23.1 ± 1.2	17.9 ± 1.1 / 23.1 ± 1.2
Coarse root biomass[8] [g dw]	.	.	.	.	5.1 ± 0.9	0.38 ± 0.09 / 0.21 ± 0.04	0.56 ± 0.06 / 0.64 ± 0.18	0.56 ± 0.06 / 0.64 ± 0.18
Fine root biomass[8] [g dw]	.	.	.	.	1.1 ± 0.4	0.17 ± 0.02 / 0.08 ± 0.01	0.41 ± 0.08 / 0.17 ± 0.05	0.41 ± 0.08 / 0.17 ± 0.05

[1] The results of Chapter 2 and 3 arose from the same experiment.
[2] The results of Chapter 5 and 6 arose from the same experiment.
[3] n. d. = not determined.
[4] The bulk density was measured in the upper 20 cm of the soil at the end of the experiment.
[5] The soil moisture could not be shown as WFPS due to the missing total pore volume.
[6] Due to the absent values for the time of planting the saplings, the values of the control treatment are listed
[7] The leaves were already enfolded at the start of the experiment.
[8] ash / beech

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THE INHIBITING EFFECT OF NITRATE FERTILISATION ON METHANE UPTAKE OF A TEMPERATE FOREST SOIL IS INFLUENCED BY LABILE CARBON

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2.1 ABSTRACT

Upland soils are the most important terrestrial sink for the greenhouse gas CH_4. The oxidation of CH_4 is highly influenced by reactive N which is increasingly added to many ecosystems by atmospheric deposition and thereby also alters the labile C pool in the soils. The interacting effects of soil N availability and the labile C pool on CH_4 oxidation are not well understood. We conducted a laboratory experiment with soil columns consisting of homogenised topsoil material from a temperate broad-leaved forest to study the net CH_4 flux under the combined or isolated addition of NO_3^- and glucose as a labile C source. Addition of NO_3^- and glucose reduced the net CH_4 uptake of the soil by 86% and 83%, respectively. The combined addition of both agents led to a nearly complete inhibition of CH_4 uptake (reduction by 99.4%). Our study demonstrates a close link between the availability of C and N and the rate of CH_4 oxidation in temperate forest soils. Continued deposition of NO_3^- has the potential to reduce the sink strength of temperate forest soils for CH_4.

Key words: *CH_4 uptake, NO_3^-, glucose, soil moisture, interaction of C and N cycles, N deposition*

2.2 INTRODUCTION

Methane (CH_4) has a 25 times higher global warming potential than carbon dioxide (CO_2). Its present concentration in the atmosphere has more than doubled from 715 to 1774 ppb since pre-industrial times over the past 150 years (Forster et al., 2007). Hence, CH_4 contributes about 15% to the present greenhouse effect of the long-lived greenhouse gases (Forster et al., 2007). Beside chemical oxidation in the troposphere (Crutzen, 1991; Denman et al., 2007), biological oxidation in aerobic soils by methanotrophs and nitrifiers represents the second strongest absorber of atmospheric CH_4 (Smith et al., 2000; Le Mer & Roger, 2001; Denman et al., 2007). In a recent study, Dutaur and Verchot (2007) calculated the global CH_4 sink of soils to 22.4 Tg yr^{-1}. The CH_4 uptake of soils in the temperate zone accounts for nearly half of this global sink (10.4 Tg CH_4 yr^{-1}). It is estimated that temperate forest soils contribute between 3 and 5.7 Tg CH_4 yr^{-1} to this sink (Curry, 2007; Dutaur & Verchot, 2007; Ishizuka et al., 2009). Therefore, any change in the CH_4 sink strength of temperate forest soils as resulting from nitrogen (N) deposition, liming or fertilisation activities, altered forest management or forest conversion is of global interest.

Over the past 200 years, temperate forest ecosystems have already received more than ten times higher anthropogenic N inputs through atmospheric deposition than in pre-industrial times (Nadelhoffer et al., 1999; Holland et al., 1999, 2005; Galloway & Cowling, 2002; Magnani et al., 2007). Many studies reported N deposition to be an important factor in the control of CH_4 uptake by forest soils (Kasimir-Klemedtsson et al., 1997; Butterbach-Bahl et al., 1998). Liu and Greaver (2009) assumed that anthropogenic N addition reduces CH_4 uptake by 3.9 to 9.1 Tg CH_4 yr^{-1} at the global scale. These figures compare well with the estimate of Dutaur and Verchot (2007) of a reduction by 17% to 40% of the CH_4 sink strength. Due to the strong impact of N addition on CH_4 uptake, intensive research in the laboratory and the field has addressed this topic (Goldman et al., 1995; Bradford, Wookey, et al., 2001; Bradford, Ineson, et al., 2001; Jang et al., 2006; Borken & Brumme, 2009; Bodelier, 2011a). In various field studies (King & Schnell, 1994a; Dobbie & Smith, 1996; Steinkamp et al., 2001) and laboratory experiments (Adamsen & King, 1993; Bender & Conrad, 1994; Priemé & Christensen, 1997; MacDonald et al., 1997), evidence was found for an inhibiting effect of ammonium (NH_4^+) fertilisation on CH_4

oxidation in forest soils. A reduction of CH_4 oxidation by NH_4^+ addition has been explained by substrate competition between NH_4^+ and CH_4 at the binding sites of the catalysing enzyme CH_4 monooxygenase (MMO) in the first step of the CH_4 oxidation pathway (Bédard & Knowles, 1989; Topp & Pattey, 1997) resulting in enhanced NH_4^+ oxidation. Other explanations are non-competitive effects exerted by the side-product hydroxylamine during NH_4^+ oxidation (Mancinelli, 1995), the production of toxic nitrite (NO_2^-) during NH_4^+ oxidation (King & Schnell, 1994b) and osmotic effects resulting from the formation of salts in the course of fertilisation (Dunfield & Knowles, 1995; Gulledge & Schimel, 1998). Furthermore, several studies reported a negative effect of oxidised N (NO_3^-) on the CH_4 oxidation in soil (Priemé & Christensen, 1997; Wang & Ineson, 2003; Xu & Inubushi, 2004; Reay & Nedwell, 2004; Ishizuka et al., 2009). This inhibiting effect has been explained by (a) the production of toxic concentrations of NO_2^- in anaerobic microsites (Adamsen & King, 1993; Whalen, 2000; Wang & Ineson, 2003) or (b) the osmotic effect of salts (Dunfield & Knowles, 1995; Gulledge & Schimel, 1998). However, the underlying mechanisms of these non-competitive inhibiting effects of oxidised N on CH_4 uptake are not yet sufficiently understood.

There is a recent debate on the influence of N addition on carbon (C) turnover and C stocks in forest soils (Magnani et al., 2007; Dezi et al., 2010; Janssens et al., 2010). According to de Vries et al. (2009), the C sequestration in European forest soils has increased as a consequence of the N deposition in the range of 5 to 23 kg C per kg N added. Thus, it is likely that a continuing high N input into forest soils will further increase the amount of labile C. There is a need to study the consequences of increasing amounts of labile C and N in forest soils and their interaction on CH_4 oxidation which is not well understood. Schnell and King (1995) studied the influences of C compounds as glucose, starch, yeast extract, methanol, ethanol, formate, acetate, malate or lactate on the CH_4 oxidation of incubated forest soil. According to their results, these C compounds neither inhibit nor stimulate CH_4 oxidation. However, the applied concentration may have not been high enough to influence the physiology and activity of the CH_4 oxidising community; moreover, the interaction with N availability was not addressed.

In this study, we focused on the effects of NO_3^- fertilisation in combination with the application of labile C (glucose) on the CH_4 uptake of a deciduous forest soil. We tested the hypotheses that (1) high NO_3^- input leads to a relevant reduction in the CH_4 uptake of the forest soil, (2) glucose neither enhances nor inhibits CH_4 uptake when added alone, but (3) in combination with NO_3^- it increases the inhibiting effect of NO_3^- on CH_4 uptake.

2.3 MATERIALS AND METHODS

2.3.1 Soil characteristics and soil sampling

The soil used for the experiment was sampled in a mixed broad-leaved temperate forest in Hainich National Park, Thuringia, Germany (51°04' N 10°30' E) and was immediately prepared for incubation. At the sampling site, the dominating tree species are *Fagus sylvatica* L., *Fraxinus excelsior* L., *Tilia cordata* Mill., *Tilia platyphyllos* Scop., *Carpinus betulus* L., *Acer pseudoplatanus* L. and *Acer platanoides* L. The soil type is a Stagnic Luvisol (IUSS Working Group WRB, 2007) of silty texture containing 1.8% sand, 80.2% silt and 18.1% clay. The sampled soil was free of carbonate (< 0.02% of C_{total}), had a pH (KCl) of 3.8 and a base saturation of 22.9%. Material of the upper 0 to 10 cm of the mineral soil was collected, excluding litter material. After collecting the soil material, it was homogenised by passing it through a 5-mm-sieve.

2.3.2 Experimental setup

For the main experiment, 16 Plexiglass cylinders (50 cm in height, 17 cm in diameter) were used and each filled with 4 kg of the freshly sieved soil. The water content at the start was 22.7% of the fresh weight. The columns were placed in a random arrangement in the laboratory. A supplementary experiment with 16 additional soil columns of the same dimensions and treated alike was conducted to (1) repeat the findings of the main experiment as well as to (2) exclude possible other effects like proposed inhibitory effect of salt addition and to (3) close the lack of control without labile C addition. Before the start of the main experiment, we kept the incubated soil for a period of 62 d under laboratory conditions to equilibrate microbial soil community to the climatic conditions and to balance the gas exchange after disturbing the natural soil structure. After this pre-experimental phase, the

experiment with two factors (addition of NO_3^- and glucose) lasted for another 62 d with three experimental phases (first, second and third N-fertilisation phase) being distinguished (days 1 – 2, 21 – 41 and 42 – 62, respectively). In the main experiment, the N treatment was replicated eight times. The effect of C addition on CH_4 was interpreted in comparison to the fluxes of the respective N treatment before C addition. At day 0 of the experiment (start of first N-fertilisation phase), day 20 (start of second N-fertilisation phase) and day 41 (start of third N-fertilisation phase), eight randomly chosen soil columns were fertilised with a KNO_3 solution in deionised water with an equivalent of 200 kg N ha^{-1}. The amount of added water was adjusted to reach a water-filled pore space (WFPS) level of 80%. On day 42 (start of third N-fertilisation phase), all 16 soil columns were additionally treated with a glucose solution (equivalent to 9419.4 kg C ha^{-1}) to simulate unlimited supply of labile C in the soil (both in the N-fertilised and the untreated control columns). The addition of N and C increased the total N (N_{total}) and organic C (C_{org}) pools in the soil columns in comparison to the initial N_{total} and C_{org} contents by absolute amounts of 7.7% and 8.0% (N) and by 33.8% and 36.0% (C) on day 42 of the experiment in the control and the N-fertilised columns, respectively.

In the supplementary experiment with fourfold replication, we examined the response of CH_4 uptake to the addition of either KNO_3 (NO_3^- source), K_2SO_4 (to test for effects of high K concentrations) or glucose (as a labile C source) using the same amounts of N, K and C (200 kg N ha^{-1}, 552 kg K ha^{-1}, 9419 kg C ha^{-1}, respectively) and the same soil and cultivation conditions as in the main experiment. Furthermore, the initial soil conditions (apart from a higher C_{org} concentration, Table 2.2) and all analytical procedures were identical to those in the main experiment. The goal of the supplementary experiment with duration of 21 d was to compare the effects of KNO_3 and K_2SO_4 and to study the effect of a labile C source independent from the NO_3^- effect. This additional experiment also served for measuring the NH_4^+ concentrations in the soil after NO_3^- addition because these data were lost in the main experiment due to technical shortcomings.

The soil columns were installed in a greenhouse with 14 h of low daylight (100 µmol m^{-2} s^{-1} PPFD; OSRAM cool white, Lightcolor 840, Munich, Germany). The water-filled pore space was measured every week by weighing the soil columns. The

pore volume and the water-filled pore space were calculated by assuming a particle density of 2.65 g cm^{-3} (Schlichting et al., 1995)and by referring to the measured soil bulk density at the experiment's beginning. The soil temperature of each soil column was measured at a depth of 7.5 cm and a horizontal distance to the column edge of 3 cm using NTC thermistors (Epcos, Munich, Germany) that were logged in 15-min intervals with a CR10 data logger (Campbell Scientific Inc., UT, USA). The soil temperature did not differ between the treatments, but decreased slightly by 3 °C over the course of the experiment (mean of 22.8 ± 0.2 °C in the N-fertilised and the control treatment).

2.3.3 Chemical soil analysis

Before the start of the experiment, we analysed five replicate samples for the chemical properties of soil (Table 2.1). The pH (KCl) was analysed in a suspension of 10 g soil in 1 *M* KCl solution using a Vario pH meter (WTW GmbH, Weilheim, Germany). The cation exchange capacity (CEC) of the soil was measured by percolating five samples of 2 g fresh soil with 0.2 *M* BaCl. The percolates were analysed with an ICP-AES (Optima 3000 XL, PerkinElmer, MA, USA). The base saturation was calculated as the percentage of base cations (Na, K, Ca and Mg) in CEC. The bulk density of the soil material in the columns was determined using steel cores with a defined volume of 100.93 cm^3. Before and after drying the soil cores at 105 °C for 24 h, the soil was weighed, and the bulk density of the dry material was calculated. At day 0 of the fertilisation experiment, the bulk density of the homogenised material was 1.05 ± 0.02 g cm^{-3}. The particle size distribution was determined with the sieving and pipette method (Schlichting et al., 1995). The concentrations of organic C (C_{org}), and total N (N_{total}) were analysed with a C/N analyzer (Vario EL, Elementar, Hanau, Germany). The concentrations of N-NO_3^- and N-NH_4^+ in mg kg^{-1} dw were measured by extracting 8 g fresh soil with 0.5 *M* K_2SO_4 solution (ratio of wet soil mass to solution, 1:3) within the following 2 to 3 h after collecting the soil. The samples were shaken for 1 h and passed through folded filters (FT-4-303-150, Sartorius Stedim, Aubagne, France). The NO_3^- and NH_4^+ concentrations of the filtered extracts were analysed by continuous flow injection colorimetry (SAN+ Continuous Flow Analyzer, Skalar Instruments, Breda, The Netherlands). The NO_3^- concentrations were determined with the copper-cadmium-

reduction method (ISO method 13395), and those of NH_4^+ with the Berthelot reaction method (ISO method 11732).

At day 0 and at the end of the experiment, soil solution was sampled from the soil column by irrigating the soil with 500 mL of distilled water. The percolating water was passed through filter papers (MN 85/70 BF, Macherey-Nagel, Düren, Germany) and the soil solution analysed with the DOC analyser (Dimatoc 100, Dimatec, Essen, Germany) to measure the concentration of dissolved organic C. The concentration of NO_3^- in the soil solution was analysed by ion chromatography (761 Compact IC, Metrohm, Herisau, Switzerland).

2.3.4 Gas flux analysis

Gas fluxes of CH_4 at the soil surface were measured three times per week in the headspace volume of soil columns. The headspace volume in the Plexiglass columns was 8.6 L. The chambers were closed for 1 h. At 0, 20, 40 and 60 min elapsed time after lid closure, gas samples were taken from the chamber headspace by flushing gas-tight 50-ml sample syringes with headspace air, using a needle and two three-way valves. The gas concentrations were analysed by a computer-controlled gas chromatographic system with a flame ionization detector for CH_4 (Shimadzu GC-14B, Kyoto, Japan). A detailed description of the gas chromatograph was given by Loftfield et al. (1997). The gas fluxes were calculated from the linear increase of gas concentration, which was measured during the chamber emplacement.

2.3.5 Data analysis

Statistical analyses were performed using SAS 9.1 software (Statistical Analysis System, SAS Institute Inc., Cary, USA). Cumulative gas fluxes were calculated by summing up all measurements for each column considering the number of measurements and the corresponding duration of the measuring phase. Frequency distributions were tested for normality with the Shapiro-Wilk test. One-way GLM with the Tukey-Kramer test was used to identify significant differences among the N-treatment means for cumulative CH_4 fluxes and soil properties showing normal distribution. Not normal-distributed soil parameters were analysed with the Wilcoxon U-test. Differences among normal-distributed CH_4 flux data of the different C treatments were assessed with the paired t-test. The Wilcoxon signed rank test was

used to identify differences between the C treatments in not normal-distributed soil parameters (this single test was carried out with the R statistical package, version 2.11.1, R Foundation for Statistical Computing, Vienna, Austria). Linear regression analysis was conducted to relate CH_4 flux to WFPS. For all analyses, significance was determined at $P < 0.05$.

2.4 RESULTS

2.4.1 Physical and chemical soil characteristics

Important chemical properties of the soil in the columns before the start of the experiment (day 0), at the end of the second N fertilisation phase (day 41) and 3 weeks after the combined application of N and C (day 62) are listed in Table 2.1. At the end of the second N fertilisation (day 41) and the end of the third experimental phase (combined addition of N and glucose, day 62), the pH (KCl) in the N-fertilised columns was significantly higher (by 0.4 and 1.1 pH units, respectively) than in the unfertilised control. The glucose application strongly increased the pH by 0.7

Table 2.1. Selected chemical parameters in the soil solution of the columns of the N-fertilised and the unfertilised control treatment in different phases of the experiment (means ± 1 SE of each eight columns).

		Unfertilised control (N0)	N-fertilised (N1)	Unfertilised control (N0)	N-fertilised (N1)
Experimental phase	Day 0	Day 41 (without glucose)		Day 62 (glucose added)	
pH(KCl)	3.82 ± 0.03	3.84 ± 0.08^{aA}	4.23 ± 0.06^{bA}	4.49 ± 0.07^{aB}	5.63 ± 0.16^{bB}
C_{org} [g kg^{-1} dw]	19.84 ± 0.44	20.44 ± 0.37^{aA}	19.20 ± 0.25^{bA}	22.99 ± 0.58^{aB}	22.62 ± 0.28^{aB}
N_{total} [g kg^{-1} dw]	1.89 ± 0.03	2.08 ± 0.03^{aA}	2.40 ± 0.08^{bA}	1.99 ± 0.01^{aA}	2.42 ± 0.09^{bA}
C:N [g g^{-1}]	10.50 ± 0.19	9.85 ± 0.10^{aA}	8.02 ± 0.17^{bA}	11.52 ± 0.18^{aB}	9.41 ± 0.30^{bB}
NO_3^- [mg N kg^{-1} dw]	6.39 ± 0.28	n. d.	n. d.	0.60 ± 0.80^{a}	35.78 ± 5.70^{b}
NH_4^+ [mg N kg^{-1} dw]	7.85 ± 0.28	n. d.	n. d.	1.53 ± 0.32^{a}	34.50 ± 4.59^{b}
DOC [mg l^{-1}]	19.4 ± 1.91	n. d.	n. d.	515.3 ± 89.9^{a}	273.2 ± 51.7^{b}
NO_3^- [mg l^{-1}]	9.6 ± 3.50	n. d.	n. d.	30.5 ± 23.6^{a}	363.35 ± 114.7^{a}

Day 0 refers to the start of the experiment; day 41 is 6 weeks after the first N fertilisation and day 62 is 3 weeks after the third N fertilisation combined with glucose addition. Lower case letters indicate significant differences between the N-fertilised and the control treatment within a given experimental phase ($P < 0.05$, Wilcoxon U-test); different upper case letters indicate significant differences between columns before and after glucose addition ($P < 0.05$, Wilcoxon signed rank test); n. d. parameter not detected.

Table 2.2. Chemical properties of the soil before (Day 0) and 21 d after the addition of KNO_3, K_2SO_4 or glucose compared with the control treatment in the supplementary experiment (means ± 1 SE, n = 4).

	Day 0	Control	KNO_3	K_2SO_4	Glucose
pH(KCl)	n. d.	4.32 ± 0.11^{a}	4.15 ± 0.09^{a}	4.12 ± 0.07^{a}	4.20 ± 0.04^{a}
C_{org} [g kg^{-1} dw]	29.20 ± 0.55	29.00 ± 0.23^{ab}	27.61 ± 0.54^{a}	29.00 ± 0.23^{ab}	32.8 ± 1.76^{b}
N_{total} [g kg^{-1} dw]	2.00 ± 0.02	1.95 ± 0.03^{a}	2.02 ± 0.05^{a}	1.96 ± 0.01^{a}	1.89 ± 0.02^{a}
C:N [g g^{-1}]	14.69 ± 0.32	14.91 ± 0.15^{a}	13.70 ± 0.16^{a}	14.80 ± 0.08^{a}	17.32 ± 0.89^{b}
N-NO_3^- [mg kg^{-1} dw]	3.27 ± 0.61	5.07 ± 3.00^{ab}	45.15 ± 2.44^{c}	12.34 ± 1.07^{b}	0.94 ± 0.81^{a}
N-NH_4^+ [mg kg^{-1} dw]	4.79 ± 0.25	3.96 ± 1.86^{a}	9.65 ± 0.85^{b}	5.29 ± 0.77^{a}	2.10 ± 1.50^{a}

Lower case letters indicate significant differences between the four treatments ($P < 0.05$, Wilcoxon U-test); n. d. parameter not detected.

(control) and 1.4 units (N-fertilised) compared with the corresponding N treatment before glucose was applied. At the 21st and 42nd day of the experiment (with the addition of N or N and C), the WFPS was adjusted to approximately 80% (Fig. 2.1a).

Subsequently, WFPS gradually declined due to soil evaporation with a slope of -1.53 ± 0.22% d^{-1} in the unfertilised soil columns and -1.53 ± 0.24% d^{-1} in the fertilised columns.

The effect of NO_3^-, glucose and K_2SO_4 on C_{org} and the mineral N concentration in the soil was investigated in the supplementary experiment (Table 2.2). The addition of KNO_3 increased the NO_3^- and NH_4^+ concentrations in the soil columns by 891% and 244%, respectively. K_2SO_4 increased the NO_3^- and NH_4^+ concentrations in the soil by 243% and 134%, respectively. Glucose led to a significant increase of C_{org} and a decline by 81% and 47% of NO_3^- and NH_4^+, respectively.

2.4.2 CH_4 fluxes

During the first phase of the main experiment, the unfertilised control columns showed enhanced uptake of CH_4 with declining WFPS. Maximum uptake (33.31 ± 1.36 µg C m^{-2} h^{-1}) was measured at day 17 when WFPS had dropped to 49.50 ± 1.64%. In contrast, NO_3^- fertilisation reduced CH_4 uptake considerably (Fig. 2.1). The CH_4 uptake of the fertilised soil remained constantly low at 3.23 ± 0.14 µg C m^{-2} h^{-1} during the first experimental phase. In the second phase of

the experiment, the maximum uptake of the unfertilised soil was 22.45 ± 0.74 µg C m^{-2} h^{-1}. In the N-fertilised soil columns, the second N application led to an even stronger inhibition of CH_4 uptake than during the first phase. The first and second NO_3^- addition significantly reduced the cumulative CH_4 uptake of the N-fertilised soil columns by 86% in the first and by 97% in the second phase compared with the unfertilised columns (Fig. 2.2). Glucose application also led to a large reduction in CH_4 uptake with average rates of only 2.41 ± 1.70 µg C m^{-2} h^{-1} during the third phase. The cumulative CH_4 uptake after glucose application was significantly lower in both the unfertilised control and in the N-fertilised columns than in the first and second experimental phases before glucose application (Fig. 2.2). The glucose addition inhibited CH_4 uptake to a similar extent (by 83%) as did the first NO_3^- addition. After combined NO_3^- and glucose addition in the third phase, the CH_4 uptake was almost completely suppressed (reduction by 99.4% compared with the uptake of the control during the first phase, Fig. 2.2).

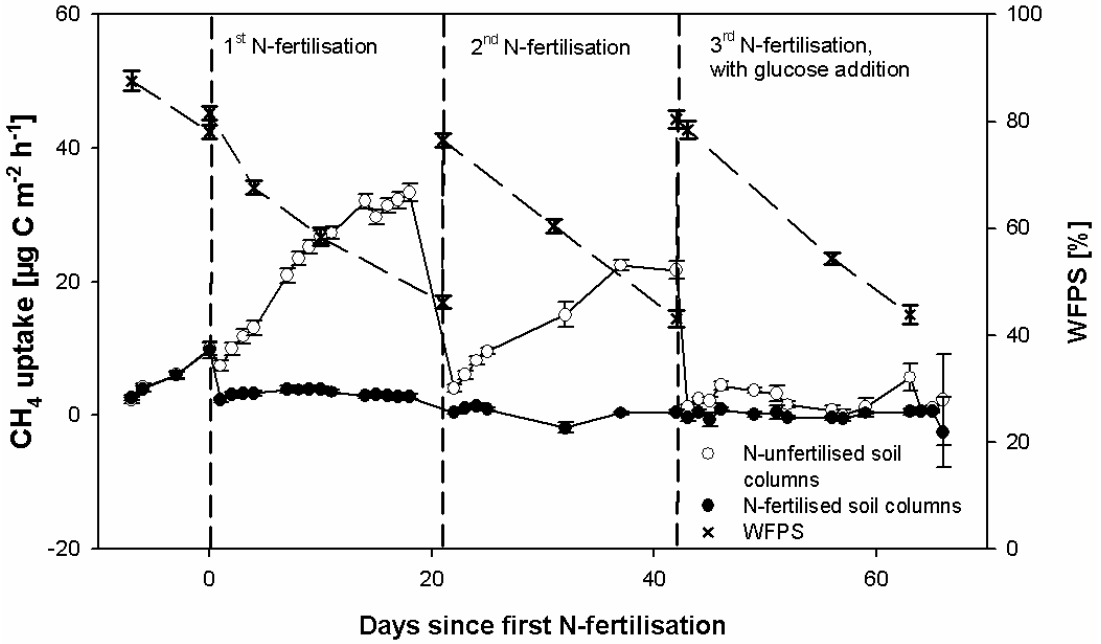

Fig. 2.1. CH_4 uptake and water-filled pore space of the soil (WFPS) in soil columns containing forest soil, either fertilised with NO_3^- or unfertilised control during the experiment of 62 d duration. Given are mean values ± 1 SE of each eight columns per N-treatment. The N-fertilization consisted of a total addition of 200 kg N ha^{-1} yr^{-1} given as KNO_3 on three occasions (first to third fertilisation). On day 41 of the experiment, all columns received additionally a glucose solution (equivalent to 9419 kg C ha^{-1}) as a labile C source.

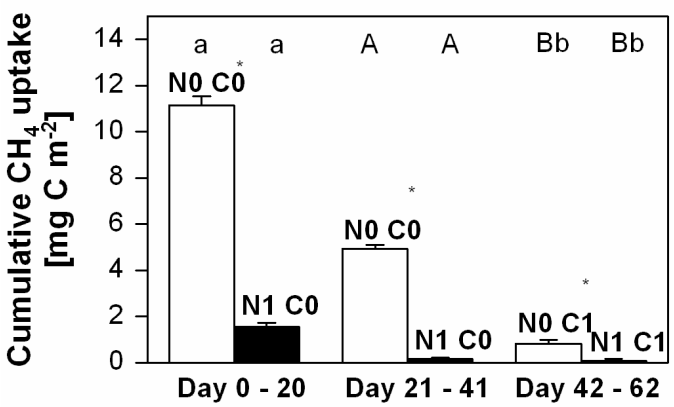

Fig. 2.2. Calculated cumulative CH_4 uptake in the different treatments during 20 d in the first, second or third phase of the experiment. Treatment acronyms are: N0 = no NO_3^- fertilisation, N1 = NO_3^- fertilisation (200 kg N ha^{-1} as KNO_3), C0 = no glucose addition, C1 = glucose addition (9419 kg C ha^{-1}). Given are means ± 1 SE (n = 8 columns per treatment). Asterisks mark significant differences between the N-fertilised and the respective control treatment for each phase ($P < 0.05$, Tukey-Kramer test), different lower case letters indicate significant differences between the first and third experimental phases for the columns of the C1 and C0 treatments, and capital letters mark such differences between the second and the third phase ($P < 0.05$, paired t-test).

In the unfertilised control treatment of the main experiment, we found a strong negative correlation between CH_4 uptake and WFPS with a large slope factor (higher CH_4 uptake at lower soil moisture) in the first and second phase of the experiment ($R^2 = 0.679$ and 0.788, respectively, Fig. 2.3). After adding NO_3^- in the fertilised treatment, this relationship had a much smaller slope (first phase $R^2 = 0.14$), or disappeared entirely (second phase). After glucose addition, no correlation between CH_4 uptake and WFPS was observed irrespective of the N treatment.

The supplementary experiment with KNO_3, K_2SO_4 or glucose addition showed after 20 d a cumulated CH_4 uptake of only 2.86 ± 0.21 mg C m^{-2} in the KNO_3 treatment, which is equivalent to about half the rate of the control (5.84 ± 1.2 mg C m^{-2}). In contrast, the columns treated with K_2SO_4 exhibited an enhanced uptake of 7.22 ± 0.18 mg C m^{-2}, which was significantly higher than the control (Fig. 2.4). The glucose application resulted in the smallest cumulated CH_4 fluxes of the three treatments (-0.04 ± 0.27 mg C m^{-2}, difference significant to the other treatments).

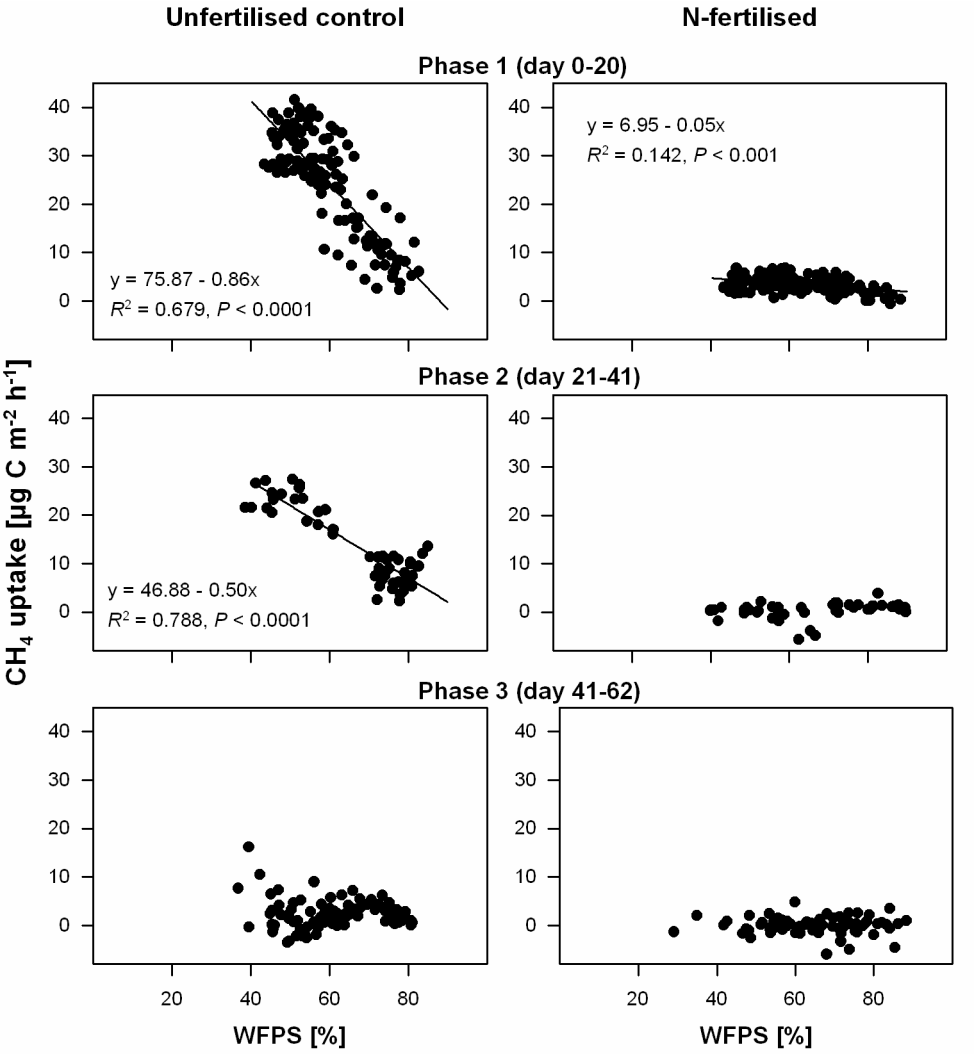

Fig. 2.3. Dependence of CH_4 uptake rate on the water-filled pore space in the soil (WFPS) in N-fertilised and unfertilised control columns in the first, second and third phase of the experiment (seven up to 14 measurements per phase in each eight columns per treatment). Nitrogen was added as 200 kg N ha^{-1} (KNO_3) at the beginning of the three phases; in the third phase, glucose (9419 kg C ha^{-1}) was additionally added as a labile C source.

2.5 DISCUSSION

Our study showed that KNO_3 relevantly inhibits the CH_4 uptake of a temperate deciduous forest soil. We found a significant reduction of the cumulative CH_4 uptake by 86% after a first addition of KNO_3 equivalent to 200 kg N ha^{-1} (10.4 µmol N g^{-1} dry soil). A second addition of the same amount of N induced a further decline to a rate of only 3% of the control (i.e. a reduction by 97%). The effect of NO_3^- on CH_4 uptake was quite variable in earlier field studies and laboratory experiments. Rigler and Zechmeister-Boltenstern (1999) found a stimulation of the CH_4 uptake of an acidic spruce forest soil after low inputs of 0.71 µmol N g^{-1} dry soil under laboratory conditions (which is a 15th of the 10.4 µmol N g^{-1} dry soil applied

in our study). The authors assumed that CH_4 oxidising bacteria may benefit from low N inputs, overcoming N limitation of bacterial growth. In contrast, repeated high inputs of a total of 140 and 530 kg $N\text{-}NO_3^-$ ha^{-1} showed no effect on CH_4 uptake of a boreal spruce forest soil under field conditions (Whalen & Reeburgh, 2000). Other field and laboratory approaches support our finding of reduced CH_4 uptake as a response to NO_3^- addition (Butterbach-Bahl et al., 1998; Reay & Nedwell, 2004; Ishizuka et al., 2009). Nitrate amendments from 0.17 to 29.96 µmol N g^{-1} to soils of temperate mixed hardwood and coniferous forests resulted in a reduction by 10 – 50% of the initial CH_4 consumption (Wang & Ineson, 2003; Xu & Inubushi, 2004). A reduction by 86% and 97% measured in our study indicates an even more pronounced inhibition of CH_4 uptake after two consecutive additions of 10.4 µmol N g^{-1} dry soil than reported in earlier studies.

Previous studies suggested that the mechanisms of CH_4 uptake inhibition in the course of NO_3^- addition are linked to (a) substrate competition at the enzyme level and (b) non-competitive effects by the formation of suppressing compounds. High concentrations of NO_2^- formed after the addition of NO_3^- are a well-studied inhibiting factor of microbial activity (Bancroft et al., 1979). Principally, the activity of NO_2^- is relatively high under alkaline pH conditions, at low temperatures or under anaerobic conditions (van Cleemput & Samater, 1995). The forest soil used in this study had a low pH value of 4.8, an average temperature of 23 °C, a moisture content of less than 80% WFPS and contained an O_2 concentration close to 210 hPa (data not shown; measured with O_2-sensitive micro-optodes, sensor type PSt1 with a resolution

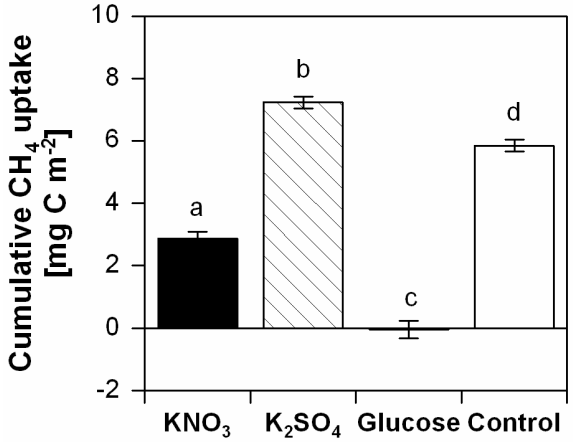

Fig. 2.4. Calculated cumulative CH_4 uptake after the addition of KNO_3, K_2SO_4 or glucose compared with the control treatment in the supplementary experiment (means ± 1 SE, n = 4). Lower case letters indicate significant differences between the four treatments ($P < 0.05$, Tukey-Kramer test).

between ± 0.1 hPa O_2 at concentrations of 2 hPa O_2 and ± 0.87 hPa O_2 at concentrations of 207 hPa O_2, with a measurement range between 0 and 500 hPa, Oxy-10 mini and Microx TX3 devices, PreSens GmbH, Regensburg, Germany). Hence, high levels of NO_2^- are not very likely as an inhibiting factor of CH_4 oxidation in our study. Several authors suggested that low osmotic potentials or salt effects caused by high cation concentrations might be another possible non-competitive inhibiting factor associated with N fertilisation (Crill et al., 1994; Hütsch et al., 1994; Bradford, et al., 2001; Bodelier, 2011). A desorption of NH_4^+ from cation exchange sites by high activities of H^+, Na^+ and K^+ cations is one possible mechanism reducing CH_4 oxidation (King & Schnell, 1998), while other authors suggested that a lowered soil water potential is responsible for the inhibiting effect of these cations on CH_4 uptake (Nesbit & Breitenbeck, 1992; Schnell & King, 1996). Wang and Ineson (2003) showed that the effect of K_2SO_4 on CH_4 uptake by a forest soil was only weak (7.0 to 56.1 µmol K g^{-1} dw), whereas the same concentration of KNO_3 strongly depressed consumption rates (7.1 to 56.2 µmol g^{-1} dw of N and K, respectively). In our supplementary experiment, CH_4 uptake remained high after the addition of 10.4 µmol K g^{-1} dw in the form of K_2SO_4, while the addition of KNO_3 with the same amount of K reduced CH_4 uptake by 51%. Thus, a co-determining effect of K on the reduction of CH_4 uptake appears unlikely. The aerobic methanotrophs are a group of methylotrophic bacteria, which are able to use CH_4 and other C1 compounds as their sole energy and C source (Trotsenko & Murrell, 2008; Dedysh & Dunfield, 2011). Recent studies also showed the existence of facultative methanotrophy in the genera *Methylocystis* (Belova et al., 2011; Im & Semrau, 2011), *Methylocapsa* (Dedysh et al., 2005; Dunfield et al., 2010) and *Methylocella* (Dedysh et al., 2005; Theisen et al., 2005). First analysis of the bacterial community within our soil columns derived from the first phase of the experiment shows the presence of the facultative methanotroph genera *Methylocystis*, as well as *Methylocella* and *Methylocapsa*, but in very low abundances (data not shown). We found *Nitrosospira* in the unfertilised soils, but not in the fertilised soil indicating another option of CH_4 oxidation using NH_4^+ monooxygenase (data not shown; Holmes et al., 1995b; Kolb et al., 2005). In our main experiment, the NH_4^+ concentrations increased by 340% compared to the initial concentration after N and C addition and consequently, fertilised columns showed significantly higher NH_4^+

concentrations than the control columns. Similarly, in our supplementary experiment, a single addition of NO_3^- resulted in by 244% higher NH_4^+ concentrations. These increases indicate that the frequently observed inhibition by NH_4^+ must also play a key role for the reduction of CH_4 uptake in our experiments. Why NO_3^- addition led to the strong increase in NH_4^+ concentrations remains unclear. Among the possible mechanisms are a stimulation of N mineralisation by the NO_3^- pulse (Kuzyakov, 2010a), the assimilation of NO_3^- in bacterial biomass and the subsequent release of NH_4^+ after cell death (Cabello et al., 2009), or perhaps dissimilatory NO_3^- reduction to NH_4^+ in anoxic micro-patches by bacteria.

In general, the WFPS and the O_2 content of the soil are recognised to be the main controlling factors of the temporal variation in CH_4 uptake (Castro et al., 1995; Bowden et al., 1998). Increased soil water content functions as a physical barrier for CH_4 diffusion into the soil, thereby reducing CH_4 uptake (Nesbit & Breitenbeck, 1992; Castro et al., 1995). In a field study under natural N supply, Guckland et al. (2009) confirmed the tight negative correlation of CH_4 uptake and WFPS in the soil of the Hainich forest, where the soil material of our experiment was collected. This negative relation was also found in our experiment under the unfertilised control conditions, but it disappeared with NO_3^- addition and its dominant effect on CH_4 oxidation (Fig. 2.3). Guckland et al. (2009) measured hourly CH_4 uptake rates of 10 to 30 $\mu g\ C\ m^{-2}\ h^{-1}$ in the National park Hainich, where our soil material was collected, during midsummer 2007, when soil temperature was 12 – 14 °C and WFPS ranged from 40% to 70%. Although in our study CH_4 uptake of the soil was not investigated under natural conditions, the same range of CH_4 uptake was observed (13 – 33 $\mu g\ C\ m^{-2}\ h^{-1}$, when WFPS ranged from 40% to 70% as well).

Our experiment evidenced not only the strong inhibiting effect of NO_3^- on CH_4 oxidation but also a negative effect by an added labile C compound. With the addition of 576 $\mu mol\ C\ g^{-1}$ dw soil in the form of glucose, the CH_4 uptake of unfertilised soil declined by 83%; adding glucose to NO_3^--fertilised soil caused a reduction by 54% of the initial cumulative CH_4 uptake under NO_3^- fertilisation in the second phase. The repression of CH_4 uptake by the combined action of added N and C was in its absolute amount even larger than the single effects of N and C. Compared with the control of the second experimental phase with no addition of N

and C, the cumulative CH_4 uptake of the soil treated with NO_3^- and glucose was reduced by 99.4%, more than the 86% by N and 83% by C addition. To our knowledge, only few studies so far have dealt with the underlying mechanisms of the effect of alternative labile C sources on CH_4 oxidation (i.e., Schnell and King 1995; Benstead et al. 1998). One possible explanation for the inhibition of CH_4 uptake by added labile C sources is the stimulation of heterotrophic microbial processes. We measured an increasing rate of N cycling after the addition of glucose, especially in the treatment with NO_3^- addition where the emission of N_2O was strongly enforced. Nitrate-reducing microorganisms must have been abundant in the soil microbial community in the N1C1 treatment while other processes such as methanotrophy were apparently suppressed. Facultative CH_4 oxidizers are capable of utilising multicarbon compounds, as acetate, succinate, pyruvate, malate or ethanol as their sole C and energy source (Dedysh & Dunfield, 2011). Thus, the obvious suppression of CH_4 uptake in the last phase of our experiment could be the consequence of a shift in preference of the methylotrophic bacteria from CH_4 to another multicarbon or C1 substrate, as acetate, pyruvate, ethanol or other side-products of glucose-utilising bacteria. The two enzymes responsible for the oxidation of CH_4 are the particulate and the soluble CH_4 monooxygenase (pMMO and sMMO). The genus *Methylocella*, owns only sMMO, which is repressed in the presence of preferred C sources as acetate, malate or other multicarbon substrates (Theisen et al., 2005). In the case of the genera *Methylocapsa* and *Methylacystis*, which prefer CH_4, pMMO and sMMO are present and not repressed in the presence of other C compounds (Dedysh & Dunfield, 2011). Finally, we cannot exclude that the apparent low CH_4 uptake rate observed in the N1C1 treatment is partly caused by enhanced CH_4 production because the addition of suitable C substrates may increase methanogenesis under anaerobic conditions (Topp & Pattey, 1997; Dalal et al., 2007; Win et al., 2010; Sasada et al., 2011). In fact, the soil moisture conditions in our experiment (40 – 80% WFPS) do not exclude the possibility that methanogenesis took place in anaerobic microsites of the not water-saturated soil (Kotiaho et al., 2010). In conclusion, our results suggest that NO_3^- and labile C compounds are agents that may significantly affect CH_4 uptake in temperate forest soils, in addition to the known factors temperature, WFPS and NH_4^+.

For the coming decades, a significant rise in temperatures and in the precipitation extremes is predicted for the temperate zone (IPCC, 2007). Another important factor will be atmospheric N deposition, which is expected to remain relatively high in large regions of Central Europe and eastern North America (Galloway et al., 2008). How these expected trends will affect the biogeochemical cycles in forest ecosystems and the chemical state of forest soils is a matter of recent discussion. Much current research focused on alterations of soil C storage and decomposition processes under changed temperature and N immission climates (Janssens et al., 2010), but the interaction between CH_4 uptake and the C and N dynamics in forest soils as schematically described in Dubbs and Whalen (2010) has not received much attention yet (Thornton et al., 2007). The suppression of the CH_4 oxidising community by increasing N may last for decades caused by the narrow and slow-growing community (Bodelier, 2011b), so that the CH_4 sink of forest soils might be changed in the long-term. For a number of structurally different forest sites in the Hainich forest, Guckland et al. (2009) reported relatively high CH_4 uptake rates in the range of 2.0 to 3.4 kg C ha^{-1} yr^{-1}. Guckland et al. (2009) detected no significant correlation between CH_4 uptake and the soil content of C_{org} and NO_3^-. However, N deposition is rather low in the region with 13 kg N ha^{-1} yr^{-1} (Mund, 2004) and complementary studies in forest stands with higher N input are needed to analyse the interaction between soil C and N availability and CH_4 oxidation under field conditions.

2.5.1 Conclusions

Our study supports the hypothesis of an inhibition of CH_4 uptake by NO_3^- in a biologically-active deciduous forest soil. The inhibition by NO_3^- was shown to be linked to increasing NH_4^+ concentrations. The hypothesis of unchanged CH_4 uptake after addition of high amounts of labile C was disproved. In contrast, we found a strong inhibiting influence of a labile C source (glucose) on CH_4 uptake. The simultaneous addition of NO_3^- and a labile C source showed that the inhibiting effects of N and C are more than additive, and the suppression of CH_4 uptake by high soil moisture contents is masked by the dominant N and C influence on this process. These findings underpin the need to investigate the interactions between the availability of NO_3^- and labile C sources on the process of CH_4 oxidation in forest

soils. Clearly, our results cannot simply be extrapolated to the field situation because we conducted our experiment with N loads > 200 kg ha^{-1}, which is much more than the 10 to 70 kg N ha yr^{-1} of N deposition measured currently in temperate European forests (Dise et al., 1998; Holland et al., 2005; Simpson et al., 2006). However, we used artificially high doses of N and labile C to demonstrate that the process of CH_4 uptake in soils is controlled by a number of additional factors that mostly have been ignored so far. Thus, this process is more difficult to predict under changing climatic and chemical conditions in future than previously thought.

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COMBINED EFFECTS OF NITRATE AND LABILE C ON THE N_2O EMISSIONS OF A TEMPERATE FOREST SOIL

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3.1 ABSTRACT

Temperate forest soils have the potential to significantly increase atmospheric N_2O concentrations when receiving high nitrogen deposition from the atmosphere. Since most of the N_2O released is a by-product of heterotrophic microbial activity, a sufficiently large labile C pool is a perquisite for elevated N_2O net release. In a two-factorial laboratory experiment we tested the combined effects of high N addition and unlimited labile C availability on the N_2O emission from a temperate deciduous forest soil. The N_2O emissions were increased eightfold after a single addition of 200 kg N ha^{-1} as KNO_3, while a second N addition resulted in no further increase. Addition of high amounts of labile C (glucose, 9419 kg C ha^{-1}) increased the cumulative N_2O emissions 18 times in N-fertilised soil and 39-fold in unfertilised soil in the 41 d following application. The combination of N and C addition led to a 70-fold increase in the N_2O emission related to untreated soil. We conclude that the currently still low N_2O emission rates that are frequently found throughout Central European forest soils, despite high atmospheric N deposition, are explained by labile C limitation of microbial activity. Our results suggest that the role of labile C and its interaction with N availability may have not been considered sufficiently in predictions of N_2O emissions from temperate forest ecosystems.

Key words: *trace gas emissions, nitrous oxide, decomposition, nitrogen availability, forest soil, organic carbon*

3.2 INTRODUCTION

The atmospheric concentration of N_2O, one of the most important anthropogenic greenhouse gases with a global warming potential 298 times larger than that CO_2, has increased by 19% since pre-industrial times (Forster et al., 2007). Main sources of N_2O are agriculture, land-use changes and biomass burning; it is predicted that globally increasing reactive nitrogen (N_r) deposition and higher temperatures will further increase the atmospheric N_2O concentrations (De Vries et al., 2006; Denman et al., 2007; Galloway et al., 2008; Janssens et al., 2010). N_2O is mainly produced by heterotrophic microorganisms that depend on the availability of labile carbon (C).

Forests are important components in the cycles of C and N on earth (Mosier, 1998; Luyssaert et al., 2010); a large part of the ecosystem C and N pools of forests is typically stored in the soil (Galloway et al., 2003; Luyssaert et al., 2010). Beside agricultural soils, upland forest soils are considered as main sources of N_2O in Europe (Butterbach-Bahl et al., 2002; Kesik et al., 2005; Jungkunst et al., 2006). However, the contribution of European forests to the global N_2O emissions is still not entirely clear (Pihlatie et al., 2005a). Nevertheless, it is undisputed that, on a global scale, forests have the greatest source potential for the expected increase in N_2O emissions in future (Reay et al., 2008).

Processes in upland soils that may lead to N release in the atmosphere are denitrification, dissimilatory nitrate reduction to ammonium (DNRA), and nitrification (Bremmer, 1997; Bateman & Baggs, 2005), all of which are biologically driven and producing N_2O as an intermediate by-product. Denitrification and DNRA are more important than nitrification in moist soil with a water-filled pore space (WFPS) > 60% (Ciarlo et al., 2008; Baggs, 2011). There is a general consensus that atmospheric N_2O originating from soils is mainly produced in the course of denitrification (Baggs, 2008). Important controlling factors of denitrification are soil aeration and soil NO_3^- concentration (Firestone & Davidson, 1989), which have been intensively studied in their effect on the N_2O emissions from soil (Fazzolari et al., 1998; Williams et al., 1998; Vor et al., 2003; Ruser et al., 2006; Ciarlo et al., 2008).

Another influential factor is the labile C availability because denitrification depends on labile C as energy source for catabolising NO_3^- (Firestone & Davidson, 1989;

Wrage et al., 2001). How different C compounds and quantities are affecting denitrification and N_2O emissions has been investigated in laboratory studies (Fazzolari et al., 1998; Murray et al., 2004; Wang et al., 2005; Henry et al., 2008; Morley & Baggs, 2010). Most studies that compared the effects of C and N addition on N_2O emissions observed a similarly large stimulation by the two agents (Weier et al., 1993; Gillam et al., 2008). However, the interaction between enhanced N and labile C availability on N_2O emissions has only rarely been studied (Azam et al., 2002; Gillam et al., 2008) and thus is still not completely understood. We are not aware of a study where the effects of added C and N compounds on the N_2O emission of a forest soil were studied under controlled laboratory conditions. More research is needed in particular for assessing the complex interacting effects of N deposition, elevated atmospheric CO_2, and higher temperatures in future climates on the N_2O emissions from forest soils. A first step is to investigate the influence of unlimited C availability and high N supply on the N_2O emissions of soil columns under controlled laboratory conditions.

While many forest soils contain relatively large organic C stocks, the amount of labile C can still be high or low (Magill & Aber, 2000; Tiunov & Scheu, 2004; Milcu et al., 2011). Under future climatic conditions, labile C might be an important regulator either mitigating or accelerating the emissions of the greenhouse gas N_2O (Murray et al., 2004; Morley & Baggs, 2010). Rising temperatures and the expected increase in atmospheric CO_2 may increase the availability of labile C for N_2O-producing organisms (Denman et al., 2007), which is mainly released to soil through rhizodeposits and in the course of heterotrophic decomposition processes (Kirschbaum, 2004; Allison et al., 2010; Kuzyakov, 2011). However, enhanced microbial activity may simultaneously reduce the pool of labile C available for N_2O production when organic matter is more rapidly decomposed to CO_2 (Blagodatskaya & Kuzyakov, 2008). It is not yet clear, how increased or reduced availability of labile C in soils is affecting the release of N_2O. If higher temperatures and N deposition increase the availability of labile C, a large increase in N_2O emissions from forest soils can be expected. If C availability is not increased or even decreased, no dramatic increase in N_2O release should occur. More available labile C has been found to promote denitrification with the possible result that a higher proportion of

nitrate (NO_3^-) is reduced to the end-product N_2 instead of the intermediate by-product N_2O (Morley & Baggs, 2010). However, N_2 is only produced when O_2 is lacking in anaerobic microsites. Climate change scenarios predict warmer summers and, for many regions of Central Europe, also reduced summer rainfall (Werner & Gerstengarbe, 2007), which makes it unlikely that hypoxia and anoxia will be more widespread in temperate forest soils in future.

We experimentally examined the influence of large amounts of added NO_3^-, simulating high atmospheric deposition scenarios, on the N_2O emissions of a temperate forest soil under conditions of potentially limited or unlimited labile C availability (glucose addition). Therefore, our study focused on the potential of C and N availability and their interactive effects to change the N_2O emissions of this soil under controlled constant soil and climatic conditions. We hypothesised that (1) even though many forest soils contain large stocks of C, the availability of labile C for N_2O emissions is limited, with the consequence that repeated high N inputs do not result in a corresponding additive increase of the N_2O release rate, and (2) high amounts of labile C in forest soils lead to similar N_2O emission rates as the application of high N amounts, as it was found in studies on non-forest soils (Weier et al., 1993; Bateman & Baggs, 2005). We further hypothesised (3) that the addition of labile C to forest soil affected by chronic N addition must result in very high N_2O emissions exceeding the sum of rates measured in soils treated with N or C sources alone.

3.3 MATERIALS AND METHODS

3.3.1 Soil characteristics and soil sampling

The soil was sampled in a mixed broad-leaved temperate forest in the Hainich National Park, Thuringia, Germany (51°04' N 10°30' E). The soil type is a Stagnic Luvisol (IUSS Working Group WRB, 2007) of silty texture composed of 1.8% sand, 80.2% silt and 18.1% clay. The soil contained no carbonate (< 0.02% of total C), 19.84 ± 0.44 g kg^{-1} dw organic C (C_{org}) and 1.89 ± 0.03 g kg^{-1} dw total nitrogen (N_{total}; Table 3.1). The pH (KCl) was 3.8 and base saturation was 22.9%. The soil material was taken from the upper 0-10 cm of the soil profile, passed through a 5 mm-sieve and homogenised before insertion into the soil columns.

3.3.2 Experimental setup

A main experiment was established with 16 acrylic glass columns (0.50 m in height, 0.17 m in diameter), to test the effect of adding two substances, (1) NO_3^- and (2) glucose. The NO_3^- (N) treatment was studied in eight replicate soil columns (eight N-treated and eight control columns). The effect of glucose (C) addition was examined in all 16 columns by comparing the measured trace gas fluxes before and after adding the C source. A supplementary two-factorial experiment with 12 additional soil columns of the same dimensions and treated alike was conducted in which N and C addition were independent treatments and not subsequently added as the C source in the main experiment (n = 4). Each of the acrylic glass cylinders was filled with 4 kg of fresh sieved soil; the water content at the experiment's start was 22.7% of the fresh weight. The soil columns were placed in a random arrangement in the laboratory. Before starting the experiment, we kept the cylinders filled with soil for 62 d under laboratory conditions. This was for allowing the soil microbial community to adapt to the new soil and climatic conditions and reach a steady-state gas exchange after disturbing the natural soil structure. The experiment was divided into four periods (day 0 – 20, 21 – 41, 42 – 62 and 63 – 83, respectively). On all three dates with N addition, the same eight randomly chosen soil columns were fertilised with a KNO_3 solution in deionised water with an equivalent of 200 kg N ha^{-1}, i.e., on day 0 of the experiment (first N application), day 20 (second N application) and day 41 (third N application). The amount of water added was adjusted to a WFPS level of 80% on day 0, day 20, 41 and 62. On day 41 (start of the third N fertilisation phase), all 16 soil columns were additionally treated with a glucose solution (equivalent to 9419 kg C ha^{-1}) to simulate unlimited supply of labile C in the soil in both the N-fertilised and the untreated control columns. The addition of N increased the N_{total} pool in the soil columns in comparison to the initial N_{total} content by 7.7% after the first N addition (measured on day 20) and 8.0% N after the second N addition (measured on day 41); the C_{org} pool increased by 33.8% and 36.0% (measured on day 62) due to the glucose addition in the control and the N-fertilised columns, respectively.

In the supplementary experiment, we examined the response of the N_2O and CO_2 fluxes to the addition of either KNO_3 (as N source) or glucose (as labile C source)

using the same amounts of N and C (200 kg N ha^{-1} and 9419 kg C ha^{-1}, respectively) compared to a control treatment each with n = 4 columns, and the same cultivation conditions as in the main experiment. Further, the initial soil conditions (except for a higher C_{org} concentration of 29.2 vs. 19.8 g C kg^{-1}, Table 3.2) and all analytical procedures were identical to those in the main experiment. Apart from separating the N and C effects, this additional experiment also served for measuring the ammonium (NH_4^+) concentrations in the soil after NO_3^- addition because these data were lost in the main experiment due to technical shortcomings.

The soil columns of both experiments were installed in a greenhouse with 14 h of daylight with low intensity (100 µmol m^{-2} s^{-1} PPFD; OSRAM cool white, lightcolor 840, Munich, Germany). The WFPS was measured every week by weighing the soil columns. The pore volume and the WFPS were calculated by assuming a particle density of 2.65 g cm^{-3} (Schlichting et al., 1995) and by referring to the measured soil bulk density at the beginning of the experiment. The soil temperature of each soil column was measured at a depth of 7.5 cm and a horizontal distance to the column edge of 3 cm using NTC thermistors (Epcos, Munich, Germany) that were logged in 15 min-intervals with a CR10 data logger (Campbell Scientific Inc., Utah, USA). The soil temperature did not differ between the treatments, but decreased by 3 °C over the course of the experiment (mean of 22.8 ± 0.2 °C in the N-fertilised and the control treatment).

3.3.3 Gas flux analysis

The fluxes of CO_2 and N_2O at the soil surface were measured three times per week in the headspace of the soil columns. The headspace volume in the acrylic glass columns was 8.6 L. The chambers were closed for 1 h. After 0, 20, 40 and 60 min, gas samples were taken from the chamber headspace by flushing gas-tight 50-ml sample syringes with headspace air, using a needle and two three-way valves. Due to extremely high CO_2 and N_2O fluxes, the time intervals had to be reduced to 1.5 min directly after C addition. The gas concentrations were analysed by a computer-controlled gas chromatographic system with a ^{63}Ni electron capture detector for CO_2 and N_2O (Shimadzu GC-14B, Kyoto, Japan). A detailed description of the gas chromatograph is given by Loftfield et al. (1997). The gas fluxes were calculated from the linear increase of the gas concentration recorded during lid closure.

3.3.4 Soil analysis

Before the start of the experiment, we analysed five soil samples for their chemical properties (Table 3.1). The pH (KCl) was measured in a suspension of 10 g soil in 1 *M* KCl solution using a Vario pH meter (WTW GmbH, Weilheim, Germany). The cation exchange capacity (CEC) of the soil was investigated by percolating 2 g fresh soil with 0.2 *M* BaCl. The percolates were analysed with an ICP-AES (Optima 3000 XL, PerkinElmer, Massachusetts, USA). The base saturation at the cation exchangers was calculated as the percentage of base cations (Na, K, Ca and Mg) in CEC. The bulk density of the soil material in the columns was determined using steel cores with a defined volume of 100.93 cm^3 and drying the soil at 105 °C for 24 hours. On day 0 of the fertilisation experiment, the bulk density of the homogenised material was 1.05 ± 0.02 $g\,cm^{-3}$. The particle size distribution was determined with the sieving and pipette method (Schlichting et al., 1995). The concentrations of C_{org} and N_{total} were analysed with a C/N analyzer (Vario EL, Elementar, Hanau, Germany). The concentrations of NO_3^- and NH_4^+ (both in mg N kg^{-1} dw) were measured by extracting 8 g fresh soil with 0.5 *M* K_2SO_4 solution (ratio of wet soil mass to solution: 1:3) directly after collecting the soil. The samples were shaken for 1 h and passed through folded filters (FT-4-303-150, Sartorius Stedim, Aubagne, France). The NO_3^- and NH_4^+ concentrations of the filtered extracts were analysed by continuous flow injection colorimetry (SAN+ Continuous Flow Analyzer, Skalar Instruments, Breda, The Netherlands). The NO_3^- concentrations were determined with the copper-cadmium-reduction method (ISO method 13395), those of NH_4^+ with the Berthelot reaction method (ISO method 11732). On day 0 and at the end of the experiment, soil solution was sampled from the soil columns by irrigating the soil with 500 mL of distilled water. The percolating water was passed through filter papers (MN 85/70 BF, Macherey-Nagel, Düren, Germany) and the soil solution was analysed with a DOC analyser (Dimatoc 100, Dimatec, Essen, Germany) for the concentration of dissolved organic C. The concentration of NO_3^- (mg L^{-1}) in the soil solution was analysed by ion chromatography (761 Compact IC, Metrohm, Herisau, Switzerland).

3.3.5 Data analysis

Statistical analyses were performed using SAS 9.1 software (Statistical Analysis System, SAS Institute Inc., Cary, USA). Cumulative gas fluxes were calculated by summing up all measurements for each column considering the number of measurements and the corresponding duration of the measuring phase. Frequency distributions were tested for normality with the Shapiro-Wilk test. One-way GLM with a Tukey-Kramer post hoc test was used to identify significant differences among the N treatment means for cumulative N_2O and CO_2 fluxes and soil properties in the datasets with normal distribution. Not normal-distributed soil parameters were analysed with the Wilcoxon U-test. Differences among normal-distributed N_2O and CO_2 flux data of the two C treatments were assessed with the paired t-test. The Wilcoxon signed rank test was used to identify differences between the C treatments in not normal-distributed soil parameters (this single test was carried out with the R statistical package, version 2.11.1, R Foundation for Statistical Computing, Vienna, Austria). For all analyses, significance was determined at $P < 0.05$.

3.4 RESULTS

3.4.1 N_2O fluxes

In the main experiment, the cumulative N_2O emissions during the first 41 d (two fertilisation periods) were significantly higher in N-fertilised soil compared to the unfertilised control (Fig. 3.1a). The first N addition led to significantly higher cumulative N_2O emissions (278.1 ± 80.0 mg N m^{-2} 20 d^{-1}, day 0 – 20) than the second N addition (38.4 ± 18.3 mg N m^{-2} 20 d^{-1}, day 21 – 41). During the first N fertilisation period, maximum N_2O effluxes of 1642.4 ± 461.6 µg N m^{-2} h^{-1} were measured, whereas the second N addition only led to maximum values of 243.7 ± 135.6 µg N m^{-2} h^{-1}. Both maximum values were recorded four d after the N application (Fig. 3.2a). During day 21 – 41, the unfertilised soil columns showed lower N_2O emissions than during day 0 – 21 as well (34.5 ± 4.9 mg N m^{-2} 20 d^{-1} vs. 5.3 ± 0.3 mg N m^{-2} 20 d^{-1}).

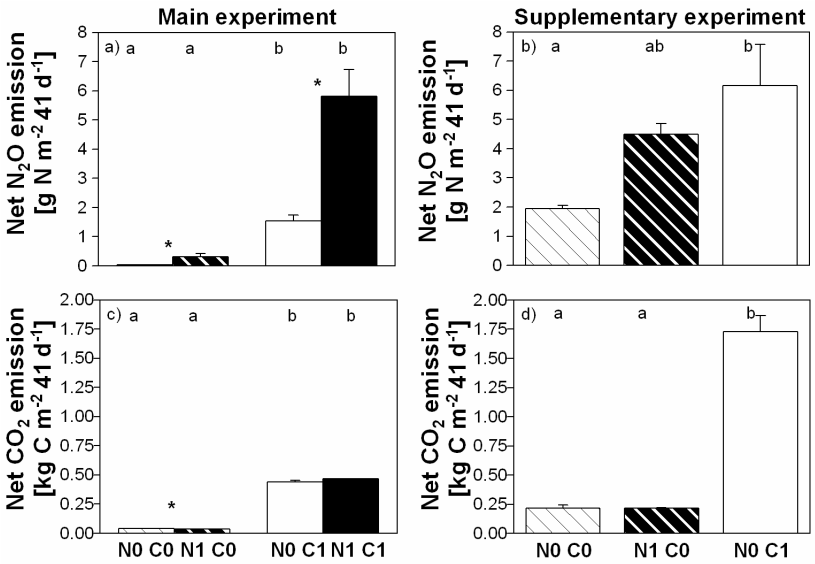

Fig. 3.1. Cumulative N_2O (a, b) and CO_2 emissions (c, d) in the four different treatments of the main experiment (a, c) or the three treatments of the supplementary experiment (b, d) in the 41 d following N and C addition. Treatment acronyms are: N0 = no nitrate fertilisation, N1 = nitrate fertilisation (200 kg N ha^{-1} as KNO_3), C0 = no glucose addition, C1 = glucose addition (9419 kg C ha^{-1}). Given are means ± 1 SE. Main experiment: n = 8 columns per treatment; asterisks mark significant differences between the N-fertilised and the respective control treatment for each phase ($P < 0.05$, Tukey-Kramer test), different lower case letters indicate significant differences between the columns of the C1 and C0 treatments ($P < 0.05$, paired t-test). Supplementary experiment: Different lower case letters indicate significant differences between the treatments (n = 4, $P < 0.05$, Tukey-Kramer test).

The application of glucose induced significantly higher cumulative N_2O emissions (between day 42 and 83) in both the unfertilised control and the N-fertilised soil than in the reference period before C addition in the main experiment (Fig. 3.1a). The combined addition of C and N compounds on day 41 resulted in significantly higher cumulative N_2O fluxes than the single C and N additions. After the addition of glucose, the unfertilised control (treatment C1N0) and the N-fertilised treatment (C1N1) reached similarly high maxima of N_2O emissions (13221.2 ± 3253.4 µg N m^{-2} h^{-1} on day 43 and 11169.8 ± 1320.4 µg N m^{-2} h^{-1} on day 49, respectively). After adding irrigation water on day 62, the N_2O emission of the N-unfertilised control remained constant at a relatively low level, whereas the emissions of the N-fertilised columns climbed to a very high maximum (24135.6 ± 4028.1 µg N m^{-2} h^{-1}, on day 65).

In the supplementary experiment, the N addition led to 2.3 times higher cumulative N_2O emissions than the control soil during the 41 d-period ($P > 0.05$, Fig. 3.1b), whereas the cumulative N_2O efflux of the soil with C addition was even significantly 3.1-fold higher than in the control soil.

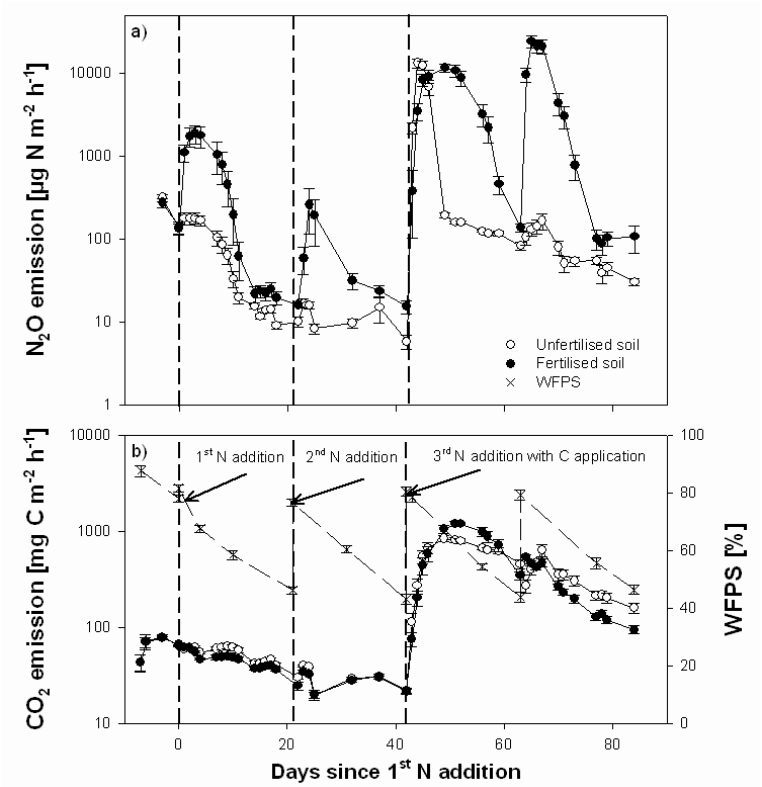

Fig. 3.2. a) N_2O emissions of the soil columns with forest soil that was either fertilised with NO_3^- or unfertilised (control) during the main experiment of 83 d duration. b) CO_2 efflux and water-filled pore space (WFPS). Given are mean values ± 1 SE of each 8 columns per N-treatment, the y axis is depicted in logarithmic scale. The N fertilisation consisted of a total addition of 200 kg N ha^{-1} yr^{-1} given as KNO_3 on three occasions (first to third fertilisation). On day 41 of the experiment, all columns received additionally a glucose solution (equivalent to 9419 kg C ha^{-1}) as a labile C source.

3.4.2 CO_2 fluxes

The glucose addition resulted in 11-fold and 13-fold increases of cumulative CO_2 emissions in the unfertilised (C1N0) control and N-fertilised treatment (C1N1), respectively (Fig. 3.1c). The cumulative CO_2 emission measured at the soil surface was significantly higher in the unfertilised soil columns (C0N0) in comparison to the fertilised columns (C0N1) during the first 41 d. For the second 41 d, N fertilisation combined with glucose addition (C1N1) increased the CO_2 emission not significantly compared to the columns without N addition (C1N0). The combined effect of C addition and N fertilisation was negligible compared to the single effect of glucose addition. After the first and second NO_3^- addition of each 200 kg N ha^{-1} (on day 0 and day 20), the CO_2 efflux tended to decrease in both treatments with time (Fig. 3.2b). The addition of glucose led to a dramatic stimulation of soil respiration with maximum peaks of CO_2 efflux of 842.0 ± 58.3 mg C m^{-2} h^{-1} (on day 49) and

1191.1 ± 51.6 mg C m^{-2} h^{-1} (on day 52) in the unfertilised control and the N-fertilised columns, respectively.

As in the main experiment, the cumulative CO_2 emissions in the supplementary experiment were significantly (6-fold) higher after adding a high concentration of labile C than in the control soil without C addition, whereas the N treatment without glucose addition led to no CO_2 flux stimulation (Fig. 3.1d).

3.4.3 Soil chemical and physical characteristics

The chemical properties of the soil in the columns before the start of the experiment (day 0), at the end of the second N fertilisation phase (day 41), and after the combined application of C and N (day 62) are listed in Table 3.1. After the second N fertilisation (day 41) and at the end of the third experimental phase (combined addition of C and N, day 62), the total N concentration in the N-fertilised columns was significantly higher (by 0.32 and 0.43 g N kg^{-1} dw, respectively) than in the unfertilised columns. After N addition, the C_{org} content of the N-fertilised soil columns was significantly lower than that of the control soil. The glucose application did not alter the N_{total} content compared to the columns' N content before glucose

Table 3.1. Selected chemical parameters in the soil solution of the columns of the N-fertilised and the unfertilised control treatment in different phases of the experiment (means ± 1 SE of each eight columns). Day 0 refers to the start of the experiment, day 41 is six weeks after the first N fertilisation and day 62 is three weeks after the third N fertilisation combined with glucose addition.

		Unfertilised control (N0)	**N-fertilised (N1)**	**Unfertilised control (N0)**	**N-fertilised (N1)**
Experimental phase	Day 0	Day 41 after N addition		Day 62 after C and N addition	
pH (KCl)	3.82 ± 0.03	3.84 ± 0.08^{aA}	4.23 ± 0.06^{bA}	4.49 ± 0.07^{aB}	5.63 ± 0.16^{bB}
C_{org} [g kg^{-1} dw]	19.84 ± 0.44	20.44 ± 0.37^{aA}	19.20 ± 0.25^{bA}	22.99 ± 0.58^{aB}	22.62 ± 0.28^{aB}
N_{total} [g kg^{-1} dw]	1.89 ± 0.03	2.08 ± 0.03^{aA}	2.40 ± 0.08^{bA}	1.99 ± 0.01^{aA}	2.42 ± 0.09^{bA}
C:N [g g^{-1}]	10.50 ± 0.19	9.85 ± 0.10^{aA}	8.02 ± 0.17^{bA}	11.52 ± 0.18^{aB}	9.41 ± 0.30^{bB}
NO_3^- [mg N kg^{-1} dw]	6.39 ± 0.28	n. d.	n. d.	0.60 ± 0.80^{a}	35.78 ± 5.70^{b}
NH_4^+ [mg N kg^{-1} dw]	7.85 ± 0.28	n. d.	n. d.	1.53 ± 0.32^{a}	34.50 ± 4.59^{b}

Lower case letters indicate significant differences between the N-fertilised and the control treatment within a given experimental phase ($P < 0.05$, Wilcoxon U-test); different upper case letters indicate significant differences between columns before and after glucose addition ($P < 0.05$, Wilcoxon signed rank test). n. d. = parameter not determined.

application. The addition of C enhanced the C_{org} content significantly by 2.55 g C kg^{-1} dw and 3.42 g C kg^{-1} dw in the unfertilised control and N-fertilised soil, respectively. The soil moisture in the columns was adjusted to 80% WFPS on day 20, 41 and 62 of the experiment and showed a more or less continuous decrease due to evaporation at a rate of -1.53% d^{-1} in all treatments to approach approximately 40% moisture content before the start of the next manipulation event (Fig. 3.2a,b).

The independent effect of NO_3^- and glucose on the NO_3^- and NH_4^+ concentrations in the soil was investigated in the supplementary experiment (Table 3.2). The NO_3^- and NH_4^+ concentrations in the soil columns increased significantly by 891% and 244%, respectively, after the addition of 200 kg N ha^{-1} in the form of KNO_3. The addition of 9419 kg C ha^{-1} (glucose) decreased the NO_3^- and NH_4^+ concentrations non-significantly by 81% and 47%, respectively.

3.5 DISCUSSION

While the first amount of NO_3^- added (200 kg N ha^{-1} equivalent to 145 mg N kg^{-1}) increased the N_2O emissions 8-fold, a same dose applied 20 d later led to no further stimulation of N_2O release despite improved NO_3^- availability. The insensitivity of the N_2O emissions to further substrate addition points toward C limitation of NO_3^- reduction in our experimental system as it was assumed in earlier studies (Weier et al., 1993; Gillam et al., 2008). Further support for our first hypothesis assuming

Table 3.2. Selected chemical parameters of the soil before (day 0) and 21 d after the addition of KNO_3 or glucose compared to the control treatment in the supplementary experiment (means ± 1 SE, n = 4).

	Day 0	Control	KNO_3	Glucose
pH (KCl)	n. d.	4.32 ± 0.11^{a}	4.15 ± 0.09^{a}	4.20 ± 0.04^{a}
C_{org} [g kg^{-1} dw]	29.20 ± 0.55	29.00 ± 0.23^{ab}	27.61 ± 0.54^{a}	32.8 ± 1.76^{b}
N_{total} [g kg^{-1} dw]	2.00 ± 0.02	1.95 ± 0.03^{a}	2.02 ± 0.05^{a}	1.89 ± 0.02^{a}
C:N [g g^{-1}]	14.69 ± 0.32	14.91 ± 0.15^{a}	13.70 ± 0.16^{a}	17.32 ± 0.89^{b}
NO_3^- [mg N kg^{-1} dw]	3.27 ± 0.61	5.07 ± 3.00^{ab}.	45.15 ± 2.44^{c}	0.94 ± 0.81^{a}
NH_4^+ [mg N kg^{-1} dw]	4.79 ± 0.25	3.96 ± 1.86^{a}.	9.65 ± 0.85^{b}	2.10 ± 1.50^{a}

Lower case letters indicate significant differences between the three treatments ($P < 0.05$, Wilcoxon U-test) n. d. = not determined.

limitation of N_2O emissions by labile C in the soil is provided by the tremendous stimulation of emissions observed after adding glucose at saturating amounts. In fact, adding 9419 kg C ha^{-1} (equivalent to 6.9 g C kg^{-1} soil) had, with a 39-fold increase in emissions compared to the control, a larger effect on N_2O emission than applying 200 kg N ha^{-1}. Thus, hypothesis 2 is falsified. Clearly, we added glucose at a saturating dose; the stimulation of N_2O emission might have been similarly large by C and N if both elements had been added in a ratio of about 20:1, simulating a biomass C/N ratio of 20. In earlier studies, the response of N_2O emission to N application ranged from no change to a fivefold stimulation of N_2O emission after KNO_3 addition at doses of 10 to 277 mg N kg^{-1} soil (Weier et al., 1993; McKenney et al., 2001; Gillam et al., 2008; Jäger et al., 2011); the largest increase in N_2O efflux was measured after adding 63.4 mg N kg^{-1} soil, i.e. half the amount added in our study (Ciarlo et al., 2008). Table 3.3 compiles the existing literature data on the effect of glucose and KNO_3^- on N_2O emissions from agricultural soils. For glucose addition, two- to tenfold increases of the N_2O emissions have been reported (Azam et al., 2002; Murray et al., 2004; Gillam et al., 2008), which was comparable to the effect of N addition (Weier et al., 1993; Bateman & Baggs, 2005). While our N effect is well in the range of literature data, this is not the case with our very large labile C effect (Table 3.3).

The relatively high soil moisture in our soil columns (40 – 80% WFPS) suggests that much of the N_2O released was produced in the course of dissimilatory processes. Fazzolari et al. (1998) assumed that DNRA dominates over denitrification when glucose and NO_3^- are added at C/N rations > 4. Thus, the glucose-C/NO_3^--N ratio of 48 in our experiment suggests that DNRA was indeed the main N_2O-producing process in the soil columns after adding N and C sources. The large increase in the soil NH_4^+ concentration after NO_3^- and glucose addition is another indication that DNRA was much more important than denitrification under these soil conditions, supporting the recently formulated assumption that the potential of DNRA to release N_2O after C and N addition may have been greatly underestimated (Morley & Baggs, 2010; Baggs, 2011).

Table 3.3. Laboratory experiments on the effect of glucose and KNO_3 addition on N_2O fluxes. Shown are the applied C and N amounts [mg kg^{-1} soil], the comparative N_2O fluxes with and without substrate addition, the initial N_{total} and C_{org} [g kg^{-1}] and NO_3^- and NH_4^+ concentrations [mg N kg^{-1}] as well as the investigated N parameters. Except in the present study, in all other studies agricultural soil was used.

Agent	Addition	N_2O flux with substrate addition	N_2O flux without substrate addition	N_{total}	C_{org}	NO_3^-	NH_4^+	Investigated N parameter	Report
N	**0, 50**	Not detected without C addition	Not detected without C addition	1.5	13.5	49.3	4.7	N_2O fluxes to detect nitrification/denitrification)	Azam et al. 2002
C	**0, 5000, 10 000**	1000 µg N h^{-1} kg^{-1} after 5000 and 10000 mg C kg^{-1} soil application at 90% H_2O content of max. water-holding capacity	0.09 µg N h^{-1} kg^{-1} at 45% H_2O content of max. water-hold. capacity						
N	**0, 63.4**	5 mg N kg^{-1} soil 22 d^{-1} at 100% WFPS	0.3 mg N kg^{-1} soil 22d^{-1} at 100% WFPS	0.81	8.6	19.9	3.6	N_2O emission and N_2O/N_2 ratio	Ciarlo et al. 2008
C	.	.	.						
N	**100**	Not detected	Not detected	.	18.9	.	.	N_2O production via DNRA in comparison to denitrifcation	Fazzolari et al. 1998
C	**250, 500, 1000**	8 mg N kg^{-1} soil 3 d^{-1} after 250 mg C addition and 50 mg N kg^{-1} 3d^{-1} soil after 1000 mg C addition at 2% O_2 concentration	Not detected						
N	**0, 10 – 1000**	Between 1.58 mg N kg^{-1} soil 5 d^{-1} after 10 mg N kg^{-1} addition and 2.65 mg N kg^{-1} soil 5 d^{-1} after 1000 mg N kg^{-1}	1.25 mg N kg^{-1} 5 d^{-1}	.	2.1	.	.	N_2O production from denitrification, N_2O/N_2 ratio	Gillam et al. 2008
C	.	.	.						experiment 1
N	**0, 10, 100**	0.05 mg N kg^{-1} soil 8 d^{-1} after 100 mg N kg^{-1} and 0.07 mg N kg^{-1} soil 8 d^{-1} after 10 mg N kg^{-1}	0.05 mg N kg^{-1} 8 d^{-1}	.	2.1	.	.	N_2O production from denitrification, N_2O/N_2 ratio	Gillam et al. 2008
C	**0, 500**	1.48 mg N kg^{-1} soil 8 d^{-1}	0.05 mg N kg^{1}8 d^{-1}						experiment 2
N	**0, 100**	0.6 mg N kg^{-1} soil 8 d^{-1}	0.1 mg N kg^{-1} soil 8 d^{-1}	.	2.1	.	.	N_2O production from denitrification, N_2O/N_2 ratio	Gillam et al. 2008
C	**0, 62.5 – 500**	Between 0.64 mg N kg^{-1} soil 8 d^{-1} after 62.5 mg C addition and 6.09 mg N kg^{-1} soil 8 d^{-1} after 250 mg C addition	0.1 mg N kg^{1-} soil 8 d^{-1}						experiment 3
N	**100**	6 mg N kg^{-1} soil 8 d^{-1}	Not detected	.	2.1	.	.	N_2O production from denitrification, N_2O/N_2 ratio	Gillam et al. 2008
C	**0, 500**	Not detected	Not detected						experiment 4
N	**0, 103, 138**	90.1 µg N m^{-2} h^{-1} at WFPS 75%	0.6 µg N m^{-2} h^{-1} at WFPS 45%	1.02	9.9	.	.	N_2O emissions during 23 and 53 d	Jäger et al. 2011
C	.	.	.						
N	**100**	.		.	21	.	.	N_2O and NO fluxes during 3 d	McKenney et al. 2001
C	**0, 500**	Between 2 and 2.5 mg N kg^{-1} d^{-1}	3 mg N kg^{-1} d^{-1}						
N	.	.		1.9	21.6	8.9	16.9	N_2O concentration of 5 d to detect DNRA, N_2O/N_2 ratio	Morley & Baggs 2010
C	**0, 28.65**	Maximum concentration of 11 mg N kg^{-1} soil after 40 hours of C and N application	Maximum concentration 11.5 mg N kg^{-1} soil						
N	**66**	Not detected	Not detected	2.8	16.7	15.3	2.4	Cumulative N_2O production	Murray et al. 2004
C	**0, 110**	9.7 mg N kg^{1} 13 d^{-1}	Cumulative N emissions of 18.7 mg kg^{-1} soil 13 d^{-1}						
N	**0, 139, 277**	Between 0.1 and 8.7 mg N m^{-2} d^{-1} in the highest N treatment measured at 75% WFPS	Between 0.1 and 4.4 mg N m^{-2} d^{-1} measured at 75% WFPS	.	.	2.5 – 89.2	0.2 – 1.8	N_2O production from denitrification during 5 d and N_2/N_2O ratio of four soils	Weier et al. 1993
C	**0, 500, 1000**	Between 5.1 and 165.4 mg N -N_2O m^{-2} d^{-1} in the highest C treatment measured at 75% WFPS	Between 1 and 44 g N ha^{-1} d^{-1} measured at 75% WFPS						
N	**145**	2.04 ± 0.59 mg N kg^{-1} soil 20 d^{-1} after the first N addition	0.25 ± 0.02 mg N kg^{-1} 20 d^{-1}	1.9	19.8	6.4	7.9		Our study
C	**6920**	12.44 ± 0.52 mg N kg^{-1} soil kg^{-1} 20 d^{-1}	0.25 ± 0.02 mg N kg^{-1} 20 d^{-1}						

The large effect of C availability on overall soil microbial activity is clearly visible from the fact that the CO_2 efflux from both the control and N fertilisation treatments were 4- to 6-fold higher in the supplementary experiment with 30% higher C_{org} concentrations than in the respective treatments of the main experiment (29.2 g C kg^{-1} soil compared to 19.8 g C kg^{-1}). However, not only soil respiration was higher in the supplementary experiment but the N_2O emissions of the control and the N treatments as well. The higher CO_2 and N_2O emissions without C addition in the supplementary experiment might be caused by more readily bio-available C in the soil compared to the main experiment.

It is known that elevated concentrations of labile C as a readily available energy source may have priming effects either on soil organic matter (SOM) mineralisation by increasing the activity of decomposing microorganisms (real priming effect) or on higher microbial metabolic activity and microbial biomass turnover (apparent priming effect; Blagodatskaya & Kuzyakov, 2008). In our study, the high C availability led to a tremendous increase in heterotrophic microbial activity as indicated by 10- and 5-fold higher CO_2 emission rates compared to the control in the main and supplementary experiments, respectively. Furthermore, the addition of glucose in the supplementary and the main experiment resulted in similarly high cumulative N_2O emissions during the 41 d-experimental period despite differences in initial C_{org} indicating that the large increase in the availability of labile C superimposed any differences in initial C availability in the soils of the two experiments. The negligible effect of the second N addition and the large increase of the N_2O and the CO_2 emissions after the C addition in the main experiment allow the conclusion that the SOM in the soil columns contained only a relatively small proportion of readily available C that could be utilised for microbial C and N turnover processes.

When our results are compared to other published data (see Table 3.3), the stimulation of N_2O emission by the first N addition is nevertheless large, despite the dominating effect of labile C in our experiment. Thus, the limitation of NO_3^- reduction by low substrate availability must have been more severe in our study than in many other experiments that were conducted with agricultural soils. For example, Gillam et al. (2008) added 1 g N kg^{-1} soil, i.e. 7 times more N than we did, and found

only a doubling of N_2O emissions compared to the control. According to pyrosequencing analysis, *Rhodanobacter* spec. occurred in significantly higher densities in N fertilised soil than in unfertilised soil after the second N addition (2.36 ± 0.52% vs. 46.87 ± 11.72% on day 41). Since these gammaproteobacteria are known to be denitrifying microorganisms, it is very likely that they largely contributed to the high N_2O emissions from the N-fertilised soil (Prakash et al., 2011; Green et al., 2012; Kostka et al., 2012).

Our data indicate that the addition of high doses of labile C and NO_3^- may act in a synergistic way on the N_2O production because combined C and N addition increased N_2O emissions 70-fold while the separate effects of C and N led only to 39- and 8-fold increases of N_2O release supporting our hypothesis (3). This is pointing to a positive interaction between labile C and NO_3^- in their effect on N_2O emission. In a laboratory experiment with silty clay, a combined NO_3^- and glucose application led to a 100 times higher emission than a glucose application alone (Azam et al., 2002). Similarly, in an experiment of eight d duration, Gillam et al. (2008) found cumulative N_2O emissions of 4.75 mg N kg^{-1} soil after combined C and N addition, whereas N addition alone led to a N_2O emission rate not higher than the unfertilised control (0.05 mg N kg^{-1} soil) and to an emission of 1.48 N kg^{-1} soil when only C was added. The latter experiment with no significant stimulation of N_2O emission by NO_3^- addition was conducted with agricultural soil where C limitation of microbial activity may have been stronger than in forest soil. Despite this difference to our study, the experiments with agricultural and forest soils have in common that labile C and N availability seem to interact and that the labile C concentration is exerting a major control on the N dynamics in the soil.

The present study aimed at characterising the potential of altering N_2O emissions from forest soils by adding C and N sources. It is clear that labile C and NO_3^- were available in concentrations that exceed the natural levels in temperate deciduous forest soils by far. However, for a better understanding of possible changes in greenhouse gas emissions under altered climate conditions, it is important to know how higher concentrations of labile C and NO_3^- would change N_2O emissions at clearly detectable levels. A likely scenario for the coming decades is that temperature, atmospheric CO_2 concentration and precipitation extremes are all

increasing on a global scale (IPCC, 2007), while atmospheric N deposition will remain high in Central Europe and eastern North America (Galloway et al., 2008). It is not yet clear whether the SOM pool of temperate forests will decrease or increase under these predicted alterations. The major fraction of SOM consists of complex recalcitrant C compounds that are difficult to catabolise. However, at higher temperature and elevated CO_2, the decomposition of complex C compounds could be accelerated (Davidson & Janssens, 2006; Allison et al., 2010; Kuzyakov, 2011). Further, the rhizodeposition of C compounds may increase under elevated CO_2 (Kuzyakov, 2011). How much labile C would be released under higher temperature and elevated CO_2 concentration, is not yet known, but it could largely exceed the current C availability. In this study, we highlighted that such an increase of labile C may offset a C limitation of N cycling in forest soils. Thus, the currently low N_2O emission rates of < 10 µg N m^{-2} h^{-1}, as they were measured under field conditions in the temperate broad-leaved Hainich forest (Guckland et al., 2010), where the soil of our experiments was collected, might substantially increase in future decades.

3.5.1 Conclusions

Our study showed that the availability of labile C can be a key factor controlling the N_2O emissions from forest soils. Additionally, our laboratory experiment gave hints on the potential of continued high N deposition in combination with higher temperatures and elevated CO_2 to largely alter N_2O emission mainly when changes in C availability in forest soils occur simultaneously. The crucial question is whether the climatic and chemical changes will increase or decrease the availability of labile C for soil microorganisms; this topic deserves further research. It is clear, however, that the role of labile C and its interaction with N availability has not been considered sufficiently enough in predictions of N_2O emissions under a variable climate. As N_2O emissions from forest soils are one of the main sources of atmospheric N_2O, the estimations done in the context of national greenhouse gas inventories after the guidelines of the IPCC (2006) should consider not only the default emission factors (the relation of N emission and N added) for agricultural sites, but must include the labile C availability and the initial mineral N status of the soil of both agricultural and forest soils as well.

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RHIZOSPHERE EFFECTS OF TREE SPECIES – LARGE REDUCTION OF N_2O EMISSIONS BY SAPLINGS OF ASH, BUT NOT OF BEECH, IN TEMPERATE FOREST SOIL

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4.1 ABSTRACT

Previous research has shown that N_2O emissions from forest soils can vary considerably with forest type. It is not yet known whether these variations are mainly reflecting differences between needle-leaved and broad-leaved forests or if there are also flux differences among different broad-leaved tree species. Furthermore, it remains unclear if these differences are merely caused by species-specific leaf litter effects, or whether root-related traits are also influential. We conducted two laboratory experiments with soil from a temperate broad-leaved forest to examine the effects of ash saplings (*Fraxinus excelsior* L.) on N_2O emissions at ambient and high nitrate availability (experiment A), and to compare the effects of ash, beech (*Fagus sylvatica* L.) and ash/beech mixture on N_2O emissions (experiment B). In both experiments, a large reduction in N_2O efflux was found for the ash treatments as compared to root-free soil (by 94% at ambient nitrate availability and by 98% after the addition of 200 kg N ha^{-1} KNO_3). The suppressing effect of ash saplings was larger than the stimulating effect of nitrate addition on N_2O emissions. Soil planted with beech saplings tended to reduce N_2O emissions as well, but the non-significant effect was much smaller than the ash effect. Our study provides evidence that species-specific rhizosphere effects can have a substantial influence on the emission of greenhouse gases from forest soils, which have to be considered in addition to leaf litter effects.

Key words: *greenhouse gas fluxes, beech, ash, tree saplings, nitrous oxide, nitrate addition*

4.2 INTRODUCTION

N_2O is an important greenhouse gas with a 298 times higher global warming potential than CO_2 and rising atmospheric concentration due to human activities (Forster et al., 2007). Upland forest soils are one major natural source of N_2O (Kesik et al., 2005), but their source strength for the global atmospheric N_2O pool is not yet fully understood (Pihlatie et al., 2005b). Consequently, there is an urgent need for a better understanding of the controlling factors of N_2O emissions from forest soils, in particular the possible effect of tree species on the soil N_2O efflux. At present, our understanding of tree species effects is mostly restricted to the finding that, in most cases, higher N_2O emissions were measured in broad-leaved than needle-leaved forests (Butterbach-Bahl et al., 1997; Butterbach-Bahl & Kiese, 2005; Ambus et al., 2006). In fact, it is not known whether temperate broad-leaved tree species are exerting significant species-specific effects on N_2O emissions from forest soils. This possibility is not unlikely because important soil parameters with a major influence on N_2O emission have been found to differ under different broad-leaved tree species (Augusto et al., 2002). For example, two field studies in a mixed deciduous broad-leaved forest in Central Germany showed higher pH as well as elevated C_{org} and N_{total} contents in the mineral soil under ash as compared to nearby beech trees (Frédéric M Holzwarth et al., 2011; Langenbruch et al., 2011). European beech (*Fagus sylvatica* L.) as the dominant tree species of the natural forest vegetation of Central Europe plays an important role in forestry. European ash (*Fraxinus excelsior* L.) coexists with beech in various forest communities as an admixed species but differs in both morphological and physiological traits from beech (Ellenberg & Leuschner, 2010; Forest Europe et al., 2011). If the two species were markedly different with respect to their soil N_2O budgets, this could well be relevant for future forestry.

N_2O emissions are driven by a variety of biogeochemical processes, but it is a consensus that most N_2O released from soils is produced in the course of denitrification (Bateman & Baggs, 2005). The main abiotic controls of denitrification are the concentrations of NO_3^- and O_2 and the quality and quantity of C sources in the soil (Firestone & Davidson, 1989). Abiotic process-level variables are regarded as stronger predictors of trace gas fluxes from the soil than biotic ecosystem-level

properties such as plant species composition (Pilegaard et al., 2006; Hopfensperger et al., 2009). Nevertheless, it has been found that biotic factors have to be considered in realistic estimates of denitrification rates (Rosswall et al., 1989; Binkley & Menyailo, 2005; Henry et al., 2008). While the functional role of trees for the belowground cycling of C and N in forest soils and species effects on denitrification are poorly understood (Kuzyakov, 2010a), it is likely that effects are exerted (1) directly via roots and (2) indirectly via leaf litter and throughfall water. Presently, nutrient fluxes with litter fall and the subsequent mineralisation processes are in the focus of most studies on the influence of trees on soil biogeochemistry (Hagen-Thorn et al., 2004; Vesterdal et al., 2008). However, rhizosphere processes related to rhizodeposition, root water and nutrient uptake, root and rhizomicrobial respiration, root decay, and physico-chemical changes in the soil as caused by root growth (Rovira, 1965; Cheng & Gershenson, 2007) are increasingly recognised as further important factors influencing C and N cycling in the soil. Given that temperate tree species often differ considerably in the morphology of their fine roots and the structure of the fine root systems (Meinen, Hertel, et al., 2009a), it is likely that different tree species may exert specific effects on the rhizosphere. How such root-mediated effects of the species are influencing the C and N dynamics of the bulk soil and the source-sink relations for CO_2 and N_2O, is of high relevance for achieving reliable estimates of the net greenhouse gas (GHG) balance of mixed and mono-specific forests.

In this study, we examined the effects of tree saplings of the early- to mid-successional species *F. excelsior* (European ash, Oleaceae) and the late-succesional species *F. sylvatica* (European beech, Fagaceae) on the N_2O emissions of a temperate broad-leaved forest soil. We established two sapling experiments (experiment A and experiment B) to quantify assumed root-mediated species effects on the release of N_2O. The two tree species differ largely with respect to fine root morphology and type of mycorrhiza: beech is characterised by thin 1st and 2nd order fine roots with many root tips that are mostly infected by fungi forming ectomycorrhizae, while ash develops relatively thick fine roots with only few root tips that form arbuscular mycorrhizae (Hölscher et al., 2002; Meinen et al., 2009). Therefore, we expected that the roots may also be different in functional terms with specific effects on the C and

N fluxes between soil and atmosphere. The effect of ash saplings on N_2O emissions was studied under ambient and excessive N availability (addition of 200 kg N ha^{-1}, experiment A) relative to a root-free control soil. In a subsequent experiment, the effects of ash saplings on the N_2O emissions were compared to the effects of beech saplings (experiment B). We tested the hypotheses that (1) ash saplings reduce the N_2O emissions from the soil significantly due to high root growth and root N uptake rates, which deplete soil N availability, while (2) the emission-reducing effect is smaller in slower growing beech. We further tested the hypothesis (3) that possible emission-reducing effects of the two tree species are small compared to the effect of increased NO_3^- availability on N_2O release.

4.3 MATERIALS AND METHODS

4.3.1 Plant and soil material

Experiment A: ash root effects on N_2O emission under ambient and excess NO_3^- availability

Tree saplings and soil were collected in a mixed deciduous broad-leaved forest in Hainich National Park, Thuringia, Germany (51°04' N 10°30' E). The soil type is a Stagnic Luvisol (IUSS Working Group WRB, 2007) of silty texture consisting of 1.8% sand, 80.2% silt and 18.1% clay. The topsoil pH (measured in a suspension with a ratio 1:5, wet soil mass to KCl solution) was 3.9. Material from the upper 0 to 10 cm of the mineral soil was collected and homogenised in the laboratory by passing it through a 5-mm sieve. The ash (*F. excelsior*) saplings were about five to seven years old. Before bud break, they were collected close to the soil sampling site in March 2010 directly before planting them into the soil columns. The saplings were carefully excavated in order to minimise disturbance of the root system. Adherent soil material was carefully removed from the roots. Five randomly chosen ash saplings were harvested to determine biometric plant parameters before the start of the experiment. The saplings had an initial shoot height of 31.6 ± 2.7 cm (mean ± 1 SE) and two to four closed leaf buds. The initial coarse and fine root biomass (dry weight) of the saplings was 5.1 ± 0.9 g and 1.1 ± 0.4 g, respectively.

Experiment B: species-specific effects of beech and ash saplings on N_2O emission

The experimental plants and the soil material were collected and treated as described for experiment A. The soil type was also a Stagnic Luvisol with silty texture being composed of 2.9% sand, 56.5% silt and 40.6% clay at a pH (KCl) of 5.3. After bud break, the three- to six-yr-old ash and beech (*F. sylvatica*) saplings were taken from the soil sampling site in May 2011 right before planting them into the soil columns. The ash and beech saplings had an initial shoot height of 19.4 ± 1.3 cm and 18.3 ± 0.8 cm, respectively, with 4 to 13 young leaves. The coarse root biomass (dry weight) of the saplings was initially 0.38 ± 0.09 g for ash and 0.21 ± 0.04 g for beech. The fine root biomass of ash and beech was 0.17 ± 0.02 g and 0.08 ± 0.01 g, respectively.

4.3.2 Experimental setup

Experiment A: ash root effects on N_2O emission under ambient and excess NO_3^- availability

A fully randomised two-factorial experiment with the presence or absence of ash saplings and N addition as independent factors was established in 16 soil columns. Four treatments were investigated, each replicated four times: A0N0 = soil incubated without ash sapling and no N addition, A0N1 = soil without sapling but treated with 200 kg N ha^{-1}, A1N0 = soil planted with an ash sapling and no N addition, A1N1 = soil planted with an ash sapling and treated with 200 kg N ha^{-1}.

The soil columns were placed in a random arrangement in a climatised greenhouse cabin. All 16 acrylic glass cylinders (Fig. 4.1) were filled with each 4 kg of freshly sieved soil (equivalent to 3.09 kg dry soil). In the eight columns with ash presence (A1), each one ash sapling was planted. After planting, the soil columns were kept under constant climatic conditions for 185 d to allow the ash plants establishing their root systems and to generate characteristic rhizosphere conditions (from day -185 to day 0). The soil columns were hold at an air temperature of 24.53 ± 0.05 °C with 14 h of low daylight (100 µmol m^{-2} s^{-1} PPFD; OSRAM cool white, lightcolor 840, Munich, Germany) and 10 h of darkness. After this pre-experimental phase, on day 0 of the experiment, the eight soil columns of the N1 treatment were fertilised with a KNO_3 solution in deionised water with an equivalent of 200 kg N ha^{-1}. The

Fig. 4.1. The soil columns made of acrylic glass used in the experiments (50 cm in height and 17 cm in diameter). Left, non-shaded (normal situation) and right, shaded columns (during gas flux measurements) are shown. Courtesy of Marco Gronwald.

investigation of species-specific effects of ash and N addition on the fluxes of CO_2 and N_2O at the soil surface was conducted in the subsequent 28 d (day 1 – 28).

The amount of water added was adjusted to reach a water-filled pore space (WFPS) level of 80% at the beginning (day 0 with N addition) and at day 14 of the experiment. In the period between day 0 and day 14, the planted soil columns (A1 treatments) were adjusted to the respective moisture level of the unplanted control (A0 treatments) for minimising differences in WFPS caused by water uptake and transpiration of the saplings. WFPS was measured two times per week by weighing the soil columns. The pore volume and the WFPS were calculated by assuming a particle density of 2.65 g cm^{-3} (Schlichting et al., 1995) and by referring to the measured soil bulk density at the beginning of the experiment. The soil temperature of each soil column was measured at a depth of 7.5 cm and a horizontal distance to the column edge of 3 cm using NTC thermistors (Epcos, Munich, Germany) that were logged in 15 min-intervals with a CR10 data logger (Campbell Scientific Inc., Utah, USA). The soil temperature did not differ between the treatments (mean of 22.96 ± 0.03 °C). The measurement of gaseous oxygen concentrations in the soil was performed with an optical method using micro-optode sensors protected by steel syringes (PSt1 sensors, equipped with an Oxy-10 mini device, PreSens GmbH, Regensburg, Germany). The tip of the syringe was placed at 7.5 cm soil depth and 4

cm off the acrylic glass wall. Before installation, the micro-optodes were calibrated in H_2O-saturated O_2-free air (N_2 atmosphere, 0 vol.-% O_2) and at atmospheric O_2 concentration (21 vol.-% O_2). The O_2 concentration was continuously measured at 5 min-intervals from day 0 to day 28 in four randomly chosen columns without ash sapling (each two in the treatments A0N0 and A0N1) and in six soil columns with a sapling (each three in the treatments A1N0 and A1N1).

Experiment B: species-specific effects of beech and ash saplings on N_2O emission

A fully randomised one-factorial experiment with four treatments was established under laboratory conditions in 18 soil columns: mono-specific columns planted either with two beech saplings or two ash saplings, mixed-species columns planted with one beech and one ash sapling and unplanted (root-free) control columns. The treatments were replicated either four times (mono-specific beech and control) or five times (mono-specific ash and mixed-species).

The columns were randomly placed in a greenhouse at 20 °C air temperature and 80% relative air humidity with 12 h of low daylight (200 µmol m^{-2} s^{-1} PPFD; EYE Lighting, Clean Ace, Mentor, OH, USA) and 12 h of darkness. Each of the eighteen acrylic glass cylinders (50 cm in height, 17 cm in diameter) was filled with 5 kg of the freshly sieved soil (equivalent to 4.70 kg dry soil). The soil columns were kept for 54 d in the greenhouse to reduce possible effects of soil disturbance and to allow for the establishment of the root systems before starting the measurements. As in experiment A, gas flux measurements were conducted for 28 days.

At day 0, WFPS was adjusted to 76%. Subsequently, WFPS was held between 60% and 76% and checked once per week by weighing the soil columns. The calculation of WFPS was done as described for experiment A.

4.3.3 Determination of soil parameters

In both experiments, 5 – 7 g soil was collected with a spatula in the upper 5 cm of the soil. In experiment A, before planting the ash saplings at day -185, at day 0 directly before N addition, and after N addition at the end of the experiment at day 28 (Table 4.1), soil was sampled; in experiment B, this was done 36 d after planting of the saplings (18 d before the start of the gas flux measurements, Table 4.2).

pH, C_{org} and N_{total} contents and salt-extractable concentrations of NH_4^+ (ammonium) and NO_3^- (nitrate) were analysed for each soil column in the upper 5 cm of the soil. The soil pH was measured in a suspension with 2 g of soil and 10 ml H_2O (pH (H_2O)), and additionally in a suspension with 2 g of soil and 10 ml of 1 M KCl solution (pH (KCl)) using a Vario pH meter (WTW GmbH, Weilheim, Germany). The particle size distribution was determined by the sieving and pipette method (Schlichting et al., 1995). The concentrations of total and organic C, and N_{total} were analysed with a C/N analyzer (Vario EL, Elementar, Hanau, Germany). The concentrations of NO_3^- and NH_4^+ were measured by extracting 3 to 5 g fresh soil with 0.5 M K_2SO_4 solution (ratio of wet soil mass to solution: 1:3). The samples were shaken for 1 h and passed through folded filters (FT-4-303-150, Sartorius Stedim, Aubagne, France). The NO_3^- and NH_4^+ concentrations of the filtered extracts were analysed by continuous flow injection colorimetry (SAN+ Continuous Flow Analyzer, Skalar Instruments, Breda, The Netherlands). The NO_3^- concentrations were determined with the copper-cadmium-reduction method (ISO method 13395), those of NH_4^+ with the Berthelot reaction method (ISO method 11732).

4.3.4 Determination of plant parameters

At the end of experiment A, the shoot length of each sapling was measured. All roots were carefully excavated from the soil, washed and cleaned from adhering soil particles. Three representative roots were digitalised on a flat-bed scanner for image analysis (Epson expression 1680, Long Beach, USA) carried out with WinRhizo 2005c software to determine specific fine root surface area (SRA, $cm^2\ g^{-1}$ dry matter), specific fine root length (SRL, cm g^{-1} dry matter), and total fine root surface area (Régent Instruments Inc., Québec, QC, Canada). Total leaf area per sapling was measured using the flat-bed scanner and the image analysis software WinFolia 2005b (Régent Instruments, Québec, Canada). The leaves, shoots and washed roots were oven-dried at 70 °C for 48 h. The dry weight of the different components was determined, and the total dry weight of each individual was calculated by adding the weight of shoot, leaves and roots. For estimating the dry matter production of the saplings, a reference dry weight of five randomly chosen saplings was obtained at the day of planting. Dry matter production was calculated by subtracting the mean dry weight of these five reference plants from the dry weight of the saplings in the soil

columns at the end of the experiment. The plant material was ground with a disc mill to determine its N concentrations (Vario EL, Elementar, Hanau, Germany). The N concentrations of shoots, leaves, and coarse and fine roots and their dry mass were used to estimate the amount of N removed from the soil by a sapling in the experimental period. In experiment B, destructive harvests could not be conducted because another experiment was subsequently conducted with these plants; only total leaf area was measured.

4.3.5 Gas flux measurements

In both experiments, the fluxes of CO_2 and N_2O between the soil surface and the atmosphere were measured three times per week in the headspace of the soil columns (between 8 and 10 a.m.), which had a volume of 8.6 L. The soil columns were closed for 1 h and shaded with a black scrim during the measurements to inhibit photosynthesis of the leaves. Every 20 min, gas samples were taken from the chamber headspace by flushing gas-tight 50 ml-sample syringes three times with headspace air, using a needle and two three-way valves. The gas concentrations were analysed by a computer-controlled gas chromatographic system with a ^{63}Ni electron capture detector (Shimadzu GC-14B, Kyoto, Japan). A detailed description of the analytical configuration of the gas chromatograph is given by Loftfield et al. 1997 (Loftfield et al., 1997). The gas fluxes were calculated from the linear increase of the gas concentration, which was measured during the chamber emplacement.

To obtain the CO_2 emissions of the soil in experiment A, leaf dark respiration was measured separately and subtracted from the total CO_2 emission. Dark respiration was measured once during the experimental phase in every ash sapling in the highest leaf with a Li-6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA) at a leaf temperature of 25 °C. In experiment A, the ash saplings of both treatments (A1N0 and A1N1) showed mean leaf dark respiration rates of 0.31 ± 0.03 µmol CO_2 m^{-2} s^{-1}. This rate was extrapolated to total leaf area and subtracted from the measured total CO_2 emissions. For estimating the leaf dark respiration of the ash saplings in experiment B, we used the mean observed rates of experiment A. For beech saplings, we assumed a mean dark respiration of 0.7 µmol CO_2 m^{-2} s^{-1}. This value is given by Rodríguez-Calcerrada et al. 2010 for the leaf dark respiration of 1.5 yr-old *Fagus sylvatica* L. seedlings at an air temperature of about 23 °C, which is close to the

established leaf temperature (25 °C) in our experiment (Rodríguez-Calcerrada et al., 2010). The gas exchange of the non-green shoots of the saplings was ignored.

4.3.6 Statistical analyses

Statistical analyses were performed using SAS 9.1 software (Statistical Analysis System, SAS Institute Inc., Cary, USA). Cumulative gas fluxes were calculated by summing up all measurements for each column considering the number of measurements and the corresponding duration of the measuring phase. Frequency distributions were tested for normality with the Shapiro-Wilk test. One-way GLM with the Tukey-Kramer test was used to identify significant differences among the N and tree treatment means for cumulative gas fluxes and soil properties if the data were normally distributed. Non normally-distributed soil parameters were analysed with the Wilcoxon U-test. Differences between the respective columns before and after N addition were detected using a Wilcoxon signed rank test. The WFPS at the nine measuring dates and the daily O_2 concentrations were tested for differences between the treatments with the Wilcoxon U-test. Since no differences were detected, neither between the WFPS of the N treatments nor between the different tree treatments, the data of all treatments were pooled for graphical presentation. The O_2 concentrations in the soil of the two N treatments did not differ significantly, and consequently, the data of the unfertilised and N-fertilised soil columns of a tree treatment were pooled as well. Linear regression analysis was conducted to relate cumulative CO_2 and N_2O fluxes to the fine root biomass or total surface area of fine root biomass at plant harvest. In all analyses, significance was determined at $P < 0.05$.

4.4 RESULTS

4.4.1 N_2O and CO_2 fluxes

Experiment A: ash root effects on N_2O emission under ambient and excess NO_3^- availability

Ash saplings reduced the cumulative N_2O emission over a 28-d period from unfertilised and NO_3^--fertilised soil compared to root-free control soil by 94% and 98%, respectively (Fig. 4.2a). In root-free control soil, NO_3^- addition (200 kg N ha^{-1}) tended to increase the cumulative N_2O emissions (differences not significant), whereas no increase upon N addition was observed in soil planted with ash. Albeit at a very low flux rate in the A1 treatments, the cumulative N_2O emissions were positively related to both the fine root surface area and the fine root biomass of the ash saplings in the columns (for both parameters: $R^2 = 0.51$, $P < 0.05$). Irrespective of the N addition, the root-free control columns showed a strong increase of N_2O emission after increasing soil moisture by irrigation up to 80% WFPS on day 0, and after a second irrigation on day 14 (Fig. 4.3a). In contrast, the N_2O emissions of soil

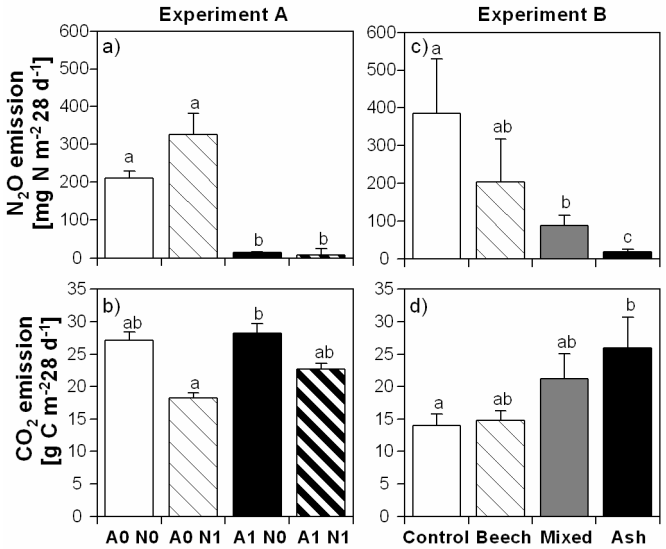

Fig. 4.2. Left column: cumulative N_2O (a) and CO_2 (b) emissions in the different tree and fertilisation treatments during 28 d (experiment A). Treatment acronyms are: N0 = no nitrate fertilisation, N1 = nitrate fertilisation (200 kg N ha^{-1} as KNO_3), A0 = no ash sapling, A1 = with ash sapling. Given are means ± 1 SE (n = 4 columns per treatment). Different lower case letters mark significant differences between the N-fertilised and the respective control treatment for each phase ($P < 0.05$, Tukey-Kramer test). Right column: cumulative N_2O (c) and CO_2 (d) emissions in the different tree treatments during 28 d (experiment B). Beech = soil columns planted with two beech saplings, Mixed = soil columns planted with one beech and one ash sapling, Ash = soil columns planted with two ash saplings (n = 4 columns per treatment in control and beech columns, and n =5 in mixed and ash columns). Different lower case letters mark significant differences between the tree treatments ($P < 0.05$, Wilcoxon U-test).

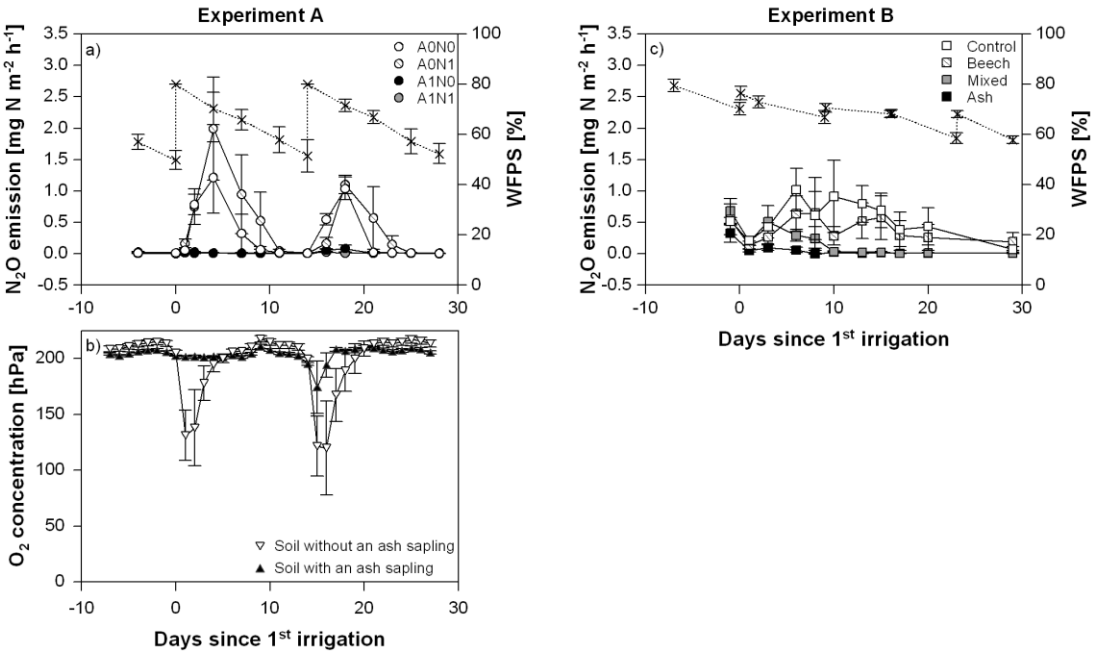

Fig. 4.3. (a) N_2O emission and water-filled pore space of the soil (WFPS) and (b) O_2 concentration (in 7.5 cm soil depth) in soil columns planted with ash saplings, either fertilised with nitrate or unfertilised (experiment A). [O_2] in unplanted soil columns is marked by open triangles, [O_2] in soil columns planted with an ash sapling with black triangles. WFPS is indicated by crosses. N0 = no KNO3 addition, N1 = KNO_3 addition, A0 = without ash sapling, A1 = with ash sapling. The N-fertilisation consisted of a total addition of 200 kg N ha^{-1} y^{-1} given as KNO_3. (c) N_2O emission and WFPS in soil columns planted with two beech, two ash, one beech and one ash sapling, or in the control columns without tree saplings (experiment B). Given are means ± 1 SE of each treatment (n = 4, except for the mixed and ash treatment in experiment B, where n =5).

planted with ash saplings remained at a low level of about 0.0127 ± 0.0033 mg N m^{-2} h^{-1} (12.7 ± 3.3 µg N m^{-2} h^{-1}) in unfertilised and 0.0109 ± 0.0011 mg N m^{-2} h^{-1} (10.9 ± 1.1 µg N m^{-2} h^{-1}) in N-fertilised soil columns, respectively.

The cumulative CO_2 emissions of soil columns planted with ash saplings were slightly, but not significantly, higher than the emission of the unplanted control in a given N treatment (Fig. 4.2b). No correlation of cumulative CO_2 emissions with fine root biomass or root surface area was detected.

Experiment B: species-specific effects of beech and ash saplings on N_2O emission

The cumulative N_2O fluxes in columns with two ash saplings were by 94% lower than the emissions of the root-free control (Fig. 4.2c). Columns with two beech saplings emitted about half the amount of N_2O as the control. Mixed beech/ash columns showed intermediate cumulative N_2O emission rates ranging between those of pure beech and ash soil columns. The soil columns with ash saplings showed low maximum N_2O emission rates (on day 3 after irrigation) that were nearly a magnitude smaller than those of the control columns (on day 6, Fig. 4.3c).

The cumulative CO_2 emission of soil planted with ash saplings was significantly higher (by 85%) than the net efflux from the control soil, whereas the CO_2 fluxes from the beech and mixed treatments were not significantly enhanced compared to the control (Fig. 4.2d). The CO_2 emission of soil planted with beech saplings showed similar CO_2 efflux rates as root-free control columns, whereas the emission of the mixed columns tended to be higher (non-significant difference).

4.4.2 Soil characteristics

Experiment A: ash root effects on N_2O emission under ambient and excess NO_3^- availability

The presence of ash saplings had significantly lowered the C/N ratio by 0.7 on day 0 (185 d after planting the saplings) and by 0.9 g g-1 (on day 28) as compared to the root-free conditions in unfertilised soil on day 0 (Table 4.1). On day 0, the NO3- concentration in the columns planted with ash saplings was up to five times higher than in the root-free soil at the time of planting, whereas on day 28, no differences between the unplanted and planted treatments could be detected. N fertilisation led to

Table 4.1 Selected chemical properties of the soil in experiment A before planting, before (day 0) and after nitrate fertilisation (day 28) of the N-fertilised (N1) and the unfertilised control treatment (N0) in dependence of the presence (A1) and absence (A0) of an ash sapling (means ± 1 SE of each four columns).

	Before planting (Day -185)	**Before N addition** (Day 0)				**After N addition** (Day 28)			
	(n = 16)	**A0 N0** (n = 4)	**A0 N1** (n = 4)	**A1 N0** (n = 4)	**A1 N1** (n = 4)	**A0 N0** (n = 4)	**A0 N1** (n = 4)	**A1 N0** (n = 4)	**A1 N1** (n = 4)
pH (H_2O)	5.02 ± 0.14	4.95 ± 0.04^{a}	5.73 ± 0.08^{b}	4.68 ± 0.06^{c}	5.36 ± 0.25^{ab}	5.37 ± 0.06^{a}	5.75 ± 0.09^{b}	5.21 ± 0.15^{a}	5.57 ± 0.18^{ab}
C_{org} [g kg^{-1} dw]	19.75 ± 0.35	18.30 ± 0.45^{a}	19.49 ± 0.62^{a}	17.67 ±0.34^{a}	19.42 ± 0.56^{a}	18.38 ± 1.03^{ab}	19.14 ± 0.26^{b}	17.93 ± 0.07^{a}	19.65 ± 0.76^{ab}
N_{total} [g kg^{-1} dw]	1.88 ± 0.02	1.76 ± 0.04^{a}	1.83 ± 0.3^{ab}	1.83 ± 0.07^{ab}	1.91 ± 0.03^{b}	1.76 ± 0.06^{a}	2.00 ± 0.056^{b}	1.89 ± 0.05^{ab}	2.08 ± 0.08^{b}
C:N [g g^{-1}]	10.47 ± 0.12	10.38 ± 0.06^{a}	10.64 ± 0.19^{a}	9.68 ± 0.17^{b}	10.77 ± 0.61^{a}	10.45 ± 0.29^{a}	9.56 ± 0.19^{b}	9.51 ± 0.25^{b}	9.49 ± 0.43^{ab}
NO_3^- [mg N kg^{-1} dw]	14.06 ± 3.67	20.15 ± 6.10^{a}	14.36 ± 3.93^{a}	132.30 ± 41.12^{b}	78.19 ± 20.59^{b}	49.10 ± 10.41^{a}	112.03 ± 12.86^{b}	62.75 ± 22.57^{ab}	121.40 ± 21.22^{b}
NH_4^+ [mg N kg^{-1} dw]	6.59 ± 1.00	5.42 ± 1.56^{a}	6.22 ± 2.45^{b}	13.69 ± 6.22^{a}	7.40 ± 4.23^{b}	3.33 ± 0.75^{a}	1.56 ± 0.26^{b}	2.58 ± 1.28^{ab}	1.55 ± 0.46^{ab}

The ash saplings were planted 185 d before N fertilisation (N addition took place on day 0). Different lower case letters indicate significant differences between the four treatments within a given experimental phase ($P < 0.05$, Wilcoxon U-test); no significant differences between the respective columns before and after N addition were detected ($P < 0.05$, Wilcoxon signed rank test)

higher pH (H2O) values, NO3- concentrations and Ntotal contents in the N1 treatment at the end of the experiment. However, the increase of the Ntotal content was less pronounced in soil columns planted with ash saplings than in bare soil. In the latter, the Ntotal content was significantly (by 14%) higher after the N addition. The N addition reduced the NH4+ concentration by half in unplanted soil, but not significantly in the soil columns with saplings. The O2 concentration in the soil planted with ash saplings tended to be slightly lower compared to the unplanted soil (Fig. 4.3b). The O2 concentration in planted columns ranged between 174.3 ± 23.6 hPa (on day 15, one day after the second irrigation) and 210.1 ± 2.2 hPa (on day 20). The variation in soil [O2] was somewhat smaller in the planted soil than in bare soil without ash saplings, where a range between 120.0 ± 41.9 hPa on day 2 and 217.1 ± 3.0 hPa on day 25 was recorded. The WFPS was adjusted to 80% on day 0 and day 14. In these two weeks, WFPS continuously declined to 51.4 ± 6.9% on day 14 (Fig. 4.3a). After the second irrigation on day 14, WFPS declined at nearly the same rate as after the first irrigation to reach a value of 52.4 ± 3.9% on day 28.

Experiment B: species-specific effects of beech and ash saplings on N_2O emission

The soil columns of experiment B that had been planted for 35 d with beech or ash saplings showed no significant differences in soil pH (H_2O) as well as C_{org} and N_{total} contents (Table 4.2). However, the saplings had a significant influence on the amount of K_2SO_4-extractable NH_4^+. In comparison to the root-free control, soil planted with

Table 4.2 Selected chemical properties of the soil 18 d before starting gas flux measurements in experiment B. The soil columns were planted with either two beech, two ash or one beech and one ash sapling or were not planted (control). Given are means ± 1 SE of each treatment.

	Control (n = 4)	**Beech** (n = 4)	**Mixed** (n = 5)	**Ash** (n = 5)
pH(H_2O)	6.13 ± 0.08[a]	6.17 ± 0.09[a]	6.21 ± 0.17[a]	6.13 ± 0.04[a]
C_{org} [g kg^{-1} dw]	18.24 ± 0.23[a]	17.85 ± 0.32[a]	17.87 ± 0.38[a]	18.36 ± 0.38[a]
N_{total} [g kg^{-1} dw]	1.75 ± 0.04[a]	1.72 ± 0.01[a]	1.71 ± 0.03[a]	1.74 ± 0.04[a]
C:N [g g^{-1}]	10.47 ± 0.26[a]	10.39 ± 0.15[a]	10.42 ± 0.14[a]	10.59 ± 0.21[a]
NO_3^- [mg N kg^{-1} dw]	39.01 ± 12.56[a]	29.24 ± 12.95[a]	20.87 ± 9.08[a]	21.59 ± 3.16[a]
NH_4^+ [mg N kg^{-1} dw]	1.54 ± 0.10[a]	1.18 ± 0.24[ab]	1.16 ± 0.12[b]	1.02 ± 0.21[b]

The trees were planted 36 d before soil analysis. Different lower case letters indicate significant differences between the four treatments ($P < 0.05$, Wilcoxon U-test).

ash saplings and the mixed columns both had a significantly lower NH_4^+ concentration. Columns planted with beech showed a slightly reduced NH_4^+ concentration as well. The salt-extractable NO_3^- concentrations were not significantly different between soil columns with saplings and the root-free control. The average WFPS of all soil columns declined from 76.5 ± 2.6% on day 0 to 59.1 ± 1.5% on day 28 (Fig 4.3c). Water added on day 9 and day 23 increased WFPS temporarily by 3.7 and 9.6%, respectively.

4.5 DISCUSSION

Our results suggest that ash saplings largely reduced N_2O emission from soil, whereas beech only slightly lowered the N_2O efflux. We are not aware of any other study on the effect of ash on N_2O emission neither under field nor laboratory conditions. However, there are some studies showing marked effects of ash on soil chemical and physical properties including bulk density, base saturation, stocks of exchangeable Ca^{2+} and Mg^{2+}, pH, N_{total}, C_{org} and C turnover (Neirynck et al., 2000; Hagen-Thorn et al., 2004; Oostra et al., 2006; Vesterdal et al., 2008, 2012; Frédéric M Holzwarth et al., 2011; Langenbruch et al., 2011; Christiansen et al., 2012). In a mixed deciduous forest of Hainich National Park (Central Germany), the mineral soil under ash trees was characterised by higher pH, and higher C_{org} and N_{total} contents than under beech trees (Frédéric M Holzwarth et al., 2011; Langenbruch et al., 2011). Holzwarth et al. 2011 (Frédéric M Holzwarth et al., 2011) assumed that differences in leaf litter quality between beech and ash are the cause of these differences in soil properties. In the present short-term laboratory study, we found evidence that 30 cm large ash saplings of 3 – 7 yr in age also significantly altered several soil characteristics such as pH and the C and N contents by a root-mediated effect independently from leaf litter input. The results suggest that NO_3^- and NH_4^+ uptake by ash roots and possibly specific root exudates are responsible for these rapid changes in soil chemistry. In a recent study, Eickenscheidt et al. 2011 (Eickenscheidt et al., 2011) estimated the contribution of leaf litter to the total annual N_2O emission with ^{15}N labelled leaf litter in a temperate beech and spruce forest in the Solling mountains, Germany. They found less than 5% of the total annual N_2O emissions being caused by N added through leaf litter decomposition with the bulk of N emitted originating from other sources. As far as we know, no field or laboratory

study on tree species effects on N_2O emission has yet attempted to separate the effects of leaf litter decomposition and root-related processes. As litter fall was excluded in our experiments, the large reduction of the N_2O emissions by up to 98% indicates that tree species can influence trace gas fluxes not only via leaf litter decomposition, but through rhizosphere processes as well, which has largely been overlooked so far. More research is needed to disentangle the effects of leaf litter decomposition and root-related processes on N_2O emissions from forest soils.

An important factor promoting N_2O emissions from soils is the existence of anoxic micro-sites in the soil. Thus, even minor differences in WFPS as were observed in our experiment (2 – 5% lower WFPS in planted compared to unplanted soil columns immediately before an irrigation event, difference not significant) could in theory influence N_2O emission rates from the soil. However, the fact that the soil in the columns planted with ash was temporarily somewhat drier than the root-free control soil, cannot explain the greatly reduced N_2O release from the ash treatment because adding water led to large increases in N_2O emissions in the control but not in the ash treatments. Thus, other factors than differences in soil aeration must be responsible for the largely different N_2O emissions from the treatments. Since WFPS mostly remained > 60 % in our experiment, it is likely that denitrification and dissimilatory NO_3^- reduction to NH_4^+ (DNRA) are responsible for the production of N_2O (Bateman & Baggs, 2005; Baggs, 2011). Roots may influence the dissimilatory reduction of NO_3^- through the local depletion of O_2 concentration by root respiration, and thereby, enhance NO_3^- reduction (Hawkes et al., 2007). The minimal O_2 concentrations of 120 ± 42 hPa (i.e. 12 vol.-% O_2) reached in unplanted soil (measured on day 16) and of 174 ± 23 hPa (18 vol.-% O_2) in planted soil were much higher than the < 5 vol.-% O_2-threshold that characterise hypoxic conditions and situations promoting the use of NO_3^- as electron acceptor (McKenney et al., 2001; Vor et al., 2003; Morley & Baggs, 2010).

Root uptake of NO_3^- can reduce nitrate availability for denitrifying organisms (Firestone, 1982). The large amount of NO_3^- added to the fertilised treatment of our experiment A must have minimised competition between the saplings and soil microbes for N. However, neither the NO_3^- concentration nor the N_{total} content were significantly lowered in soil planted with ash saplings (Table 4.1) and the large

reduction in N_2O emission by ash persisted under conditions of optimal NO_3^- availability. From the N concentration in plant biomass we estimated that during the experiment the ash saplings took up about 1.3% of the total N stored in the soil prior to planting, irrespective of the N treatment, whereas the addition of N resulted in the increase of the N_{total} content by about 8% in the N-fertilised treatment. We conclude that N uptake by the ash roots and their effect on NO_3^- availability cannot be the only reason for the dramatic decline in N_2O emissions from the soil columns with ash saplings.

In contrast to the inhibiting effect of ash roots, soil with beech saplings showed more than 10 times higher cumulative N_2O emissions than the ash treatment. Further, the flux was subject to a considerable temporal variation during the 28 d of the experiment, similar to the root-free control soil. In a field study in temperate beech and spruce forests, high N_2O emissions up to 150 µg N m^{-2} h^{-1} with large temporal variation were measured beneath beech trees, whereas the emissions remained relatively constant at rates < 20 µg N m^{-2} h^{-1} close to spruce trees (Klaus Butterbach-Bahl et al., 2002). Apart from possible effects of leaf litter quality and N release from decomposing litter, another reason for higher N_2O emissions from the soil in beech forests could perhaps be species-specific morphological and/or physiological attributes of the fine roots of European beech compared to spruce (Silver et al., 2005).

The correlation between measured N_2O emissions and the fine root biomass and fine root surface area of the ash saplings in our study supports the assumption that fine root mass and its activity is controlling N_2O emissions in the case of ash. The importance of root biomass and its morphology seems to point at a role of rhizodeposits, when attempting to explain the strong negative ash, but not beech, effect on N_2O release. It has been found that low molecular-weight C compounds released from roots can promote NO_3^- reducing processes in the rhizosphere (Henry et al., 2008). These molecules seem to serve as immediate C and energy sources for denitrifying microorganisms and they are enhancing decomposition, which additionally increases the labile C pool (Cheng & Kuzyakov, 2005; Henry et al., 2008; Kuzyakov, 2010a). Thus, one possible explanation of the ash root effect is that root activity has increased the overall availability of labile C, which allowed

denitrifiers to reduce more NO_3^- completely to N_2 than just to N_2O. Alternatively, specific exudates of ash roots could have inhibited biological activity as it has been found in other plant species (Brimecombe et al., 2007). Recently, Blagodatskaya et al. 2010 (Blagodatskaya et al., 2010) found a strong relation between the fungal-to-bacterial respiratory ratio and N_2O production with a higher N_2O efflux at a broader fungal-to-bacterial ratio. Similarly, measurements conducted in close relation to our experiment showed a larger fungal-to-bacterial ratio in the soil under beech saplings as compared to the ash treatment that was linked to the much higher N_2O emissions observed under beech, even though the soil bacterial community was more or less similar between the two treatments (S. Cesarz unpublished data, Fender, et al. 2013). Thus, the reported differences in N_2O emissions might indeed be linked to differences in the fungal-to-bacterial ratio. The N_2O production by fungal denitrification is widely accepted (Baggs, 2011). More recently, it was shown that ectomycorrhizal fungi are able to produce N_2O (Prendergast-Miller et al., 2011). We found no study, which detected the link between N_2O production and arbuscular mycorrhiza. In a current study, the N_2O production in soils by denitrifying fungi was related to the formate concentration in soil (Ma et al., 2008) and Fender et al. (2013) found significantly larger formate concentrations in the proximity of beech compared to ash roots. These higher formate concentrations might induce higher N_2O production by ectomycorrhizal as well as saprotrophic fungi in soil columns with beech. However, too little is known about qualitative and quantitative differences in rhizodeposits released from beech and ash roots to decide between the promoting and inhibiting effects of rhizodeposits. Further studies on the specific nature and function of rhizodeposition of the roots of different tree species are needed for a better understanding of how tree roots are influencing the processing of C and N in the rhizosphere.

4.5.1 Conclusions

In both N availability scenarios of experiment A and experiment B, ash saplings led to a large reduction of N_2O emissions from the soil, thus, partly supporting our hypothesis (1) that ash saplings reduce the N_2O emissions due to high N uptake rates. However, compared to the root-free control soil, ash roots did not significantly reduce the NO_3^- concentration and N_{total} content of the soil, which suggests that other factors than NO_3^- depletion due to root uptake must have caused the dramatic reduction of N_2O emission, which partly falsifies our hypothesis (1). The reducing effect of ash saplings on the N_2O emission was large (94% reduction compared to the root-free control) in comparison to the effect of NO_3^- addition (54% increase in root-free soil), which allows rejecting hypothesis (3). In fact, the promoting effects of NO_3^- addition and the increase in WFPS were completely masked by the inhibiting effect of ash roots on N_2O emission. In contrast to the strongly reduced N_2O emission by the ash rhizosphere (always < 100 µg N m^{-2} h^{-1}), beech saplings only slightly lowered the N_2O emission (maxima > 500 µg N m^{-2} h^{-1} were observed) compared to the root-free control. This finding confirmed our hypothesis (2). In our two experiments, the net release of CO_2 under standard conditions was higher from soil planted with ash saplings than from root-free control soil or soil planted with similar-sized beech saplings. In the light of the greenhouse gas budget, these higher CO_2 emissions were more than balanced by the considerably smaller N_2O emissions when expressed as CO_2 equivalents (calculated by multiplying the cumulative N_2O fluxes of 28 d with the global warming potential of N_2O of 298 (Forster et al., 2007), Table 4.3). Calculating CO_2 equivalent totals for the experiments A and B revealed by 50 and 33% lower CO_2-eq emissions from soil planted with ash as compared to root-free control soil and soil planted with beech, respectively. We conclude that, beside the well-studied effects of tree leaf litter, it is highly important to consider species-specific root effects on greenhouse gas (GHG) fluxes from forest soils as well in order to achieve more reliable GHG inventories for temperate forests. If our laboratory results on the low N_2O emissions under ash are confirmed by field studies and under a broader set of environmental conditions, this apparent functional species characteristic has the potential to influence tree species selection in Central European forestry in future.

Table 4.3. CO_2 equivalents [CO_2-eq g m^{-2} 28 d^{-1}] calculated for the CO_2 and N_2O fluxes from the soil columns of experiment A and B.

	Experiment A				Experiment B			
	A0N0 (n = 4)	**A0N1 (n = 4)**	**A1N0 (n = 4)**	**A1N1 (n = 4)**	**Control (n = 4)**	**Beech (n = 4)**	**Mixed (n = 5)**	**Ash (n = 5)**
CO_2	99.4 ± 9.5^{a}	66.8 ± 31.7^{b}	113.3 ± 8.9^{a}	83.1 ± 7.1^{ab}	52.0 ± 8.3^{a}	57.9 ± 5.5^{a}	77.8 ± 14.1^{a}	95.0 ± 17.6^{a}
N_2O	98.7 ± 18.1^{ab}	152.5 ± 4.4^{a}	34.3 ± 27.3^{b}	3.8 ± 7.0^{b}	180.7 ± 67.6^{a}	119.4 ± 61.1^{ab}	41.3 ± 13.2^{b}	8.9 ± 3.3^{c}
Total	198.1 ± 17.4^{a}	219.3 ± 31.7^{a}	147.6 ± 31.6^{ab}	86.8 ± 7.1^{b}	232.7 ± 74.5^{a}	177.3 ± 58.0^{a}	119.1 ± 20.6^{a}	103.9 ± 16.5^{a}

Experiment A: N0 = no KNO_3 addition, N1 = KNO_3 addition, A0 = without ash sapling, A1 = with ash sapling. The N-fertilisation consisted of a total addition of 200 kg N ha^{-1} y^{-1} given as KNO_3.
Experiment B: Control = soil columns without saplings, Beech = soil columns planted with two beech saplings, Mixed = soil columns planted with one ash and one beech sapling, Ash = soil columns planted with two ash saplings.
Given are means ± 1 SE of the treatments. Within each experiment, significant differences are marked by different lower case letters ($P < 0.05$, experiment A: Tukey-Kramer test, experiment B: Wilcoxon U-test).

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ROOT-INDUCED TREE SPECIES EFFECTS ON THE SOURCE/SINK STRENGTH FOR GREENHOUSE GASES (CH_4, N_2O AND CO_2) OF A TEMPERATE DECIDUOUS FOREST SOIL

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5.1 ABSTRACT

Through their leaf litter and throughfall water, tree species can have a pronounced influence on soil chemistry. However, there is little knowledge of species-specific root effects on greenhouse gas fluxes between forest soils and the atmosphere. By growing saplings of beech (*Fagus sylvatica*) and ash (*Fraxinus excelsior*) in monoculture or mixture at defined atmospheric and soil conditions in rhizotrons, we tested four hypotheses related to potential root-induced tree species effects on the uptake of CH_4 and the emission of N_2O and CO_2 from the soil. This design excluded putative effects of leaf litter mineralisation on trace gas fluxes. Gas fluxes were measured biweekly using the closed chamber technique; the CO_2 derived from root respiration was estimated, and the concentration of organic acids in the rhizosphere solution was analysed. Rhizotrons planted with ash took up significantly more CH_4 and emitted less N_2O than control rhizotrons without plants. CH_4 and N_2O fluxes from beech rhizotrons did not differ from the root-free control but were significantly smaller (CH_4) or higher (N_2O) than the fluxes from the ash treatment. While root respiration of ash was higher than of beech, root-induced soil respiration was higher in the rhizosphere of beech roots. The concentration of organic acids tended to be higher in the rhizosphere of beech and also the composition was different from that of ash. We conclude that tree species identity may substantially alter the soil source/sink strength for greenhouse gases through root-related processes.

Key words: *Fagus sylvatica, Fraxinus excelsior, greenhouse gas exchange, methane oxidation, organic acids; root growth*

5.2 INTRODUCTION

The net greenhouse gas (GHG) balance of European forest soils was recently estimated at -19 ± 11 g Ceq-CO_2 m^{-2} yr^{-1}, indicating an on average higher net uptake of GHG by forest soils than by grasslands, peatlands, and croplands (Schulze et al., 2010). Regarding CH_4, temperate forest soils contribute with an estimated amount of 3 – 5.7 Tg CH_4 yr^{-1} to the most important terrestrial sink for atmospheric CH_4, i.e. the oxidation of methane in soils (Curry, 2007; Dutaur & Verchot, 2007; Ishizuka et al., 2009). In contrast, temperate forest soils typically are sources of atmospheric N_2O (Kesik et al., 2005). Due to the complex source/sink function of soils in the global C and N cycles, specific interest is currently paid to the quantification of gas fluxes between the soils of deciduous forests and the atmosphere, and how they can be influenced for mitigating global warming.

Recent studies found a significant tree species effect on the CH_4, N_2O and CO_2 fluxes from the soils of European deciduous forests (Borken & Beese, 2006; Degelmann et al., 2009). The CH_4 uptake was found to be higher in beech-dominated stands compared to stands of spruce (Klaus Butterbach-Bahl et al., 2002). Comparative studies on the N_2O emission from forest soils reported predominantly higher effluxes in broad-leaved than needle-leaved forests (Butterbach-Bahl & Kiese, 2005; Ambus et al., 2006). Regarding methane Guckland et al. (2009) detected no difference in the CH_4 fluxes of the soil among forest patches with varying abundance of beech in a mixed broad-leaved deciduous forest. Whether GHG exchange is dependent on the species identity of broad-leaved trees, is not well known. For example, Vesterdal et al. (2012) measured significantly higher CO_2 emissions under 30-year old ash than under beech trees in a common garden experiment with six broad-leaved tree species, but we are not aware of comparative studies on the effects of beech and ash on N_2O and CH_4 fluxes.

The bulk of abiotic factors affecting the GHG exchange between soil and atmosphere are well studied, among them soil temperature, soil bulk density, soil acidity, soil moisture and N deposition (Davidson et al., 1998; Smith et al., 2000; Le Mer & Roger, 2001; Jungkunst & Fiedler, 2007; Bagherzadeh et al., 2008; Ciarlo et al., 2008). More recently, the influences of biotic factors such as tree species identity, and the activity of soil fauna, fungi and the soil microbial community on soil

processes, that may affect gas fluxes, have received increased attention (Binkley & Menyailo, 2005). Nevertheless, it is not well understood how changes in the biotic components of forest ecosystems affect GHG fluxes (Hanson et al., 2000; Silver et al., 2005; Paterson et al., 2009b).

It is undisputed that the effects of trees on the C and N cycling in the soil are larger than that of most other organism groups (Brady & Weil, 2002). The influence has been explained by changes in physical and chemical properties of the soil as a consequence of litter input, stemflow, throughfall and root activity (Menyailo & Huwe, 1999; Erickson et al., 2002; Guckland et al., 2009; van Haren et al., 2010). In most cases, tree species effects on CH_4 uptake or N_2O release have been thought to occur mainly through the input of litter and its specific properties (Hagen-Thorn et al., 2004; Vesterdal et al., 2008).

However, it is clear that roots may influence the soil via exudates, the depletion of water and nutrient reserves, decaying root material, respiration and physical changes caused by root growth and root architecture (Rovira, 1965; Cheng & Gershenson, 2007). These processes may stimulate or inhibit certain groups of biota and biochemical processes in the soil (Fig. 5.1). For example, in a comparative field study in a deciduous broad-leaved forest in Central Germany (Hainich forest), higher pH and higher contents of organic carbon (C_{org}) and total nitrogen (N_{total}) were found in the mineral soil under ash trees as compared to profiles under nearby beeches (Frédéric M Holzwarth et al., 2011; Langenbruch et al., 2011). From such field studies it is usually difficult to establish causal links between observed differences in gas fluxes under different tree species and tree functional traits (Klaus Butterbach-Bahl et al., 2002; von Arnold et al., 2005; Vesterdal et al., 2008; Christiansen & Gundersen, 2011). Thus, our knowledge about tree species-specific rhizosphere effects on greenhouse gas fluxes is very poor.

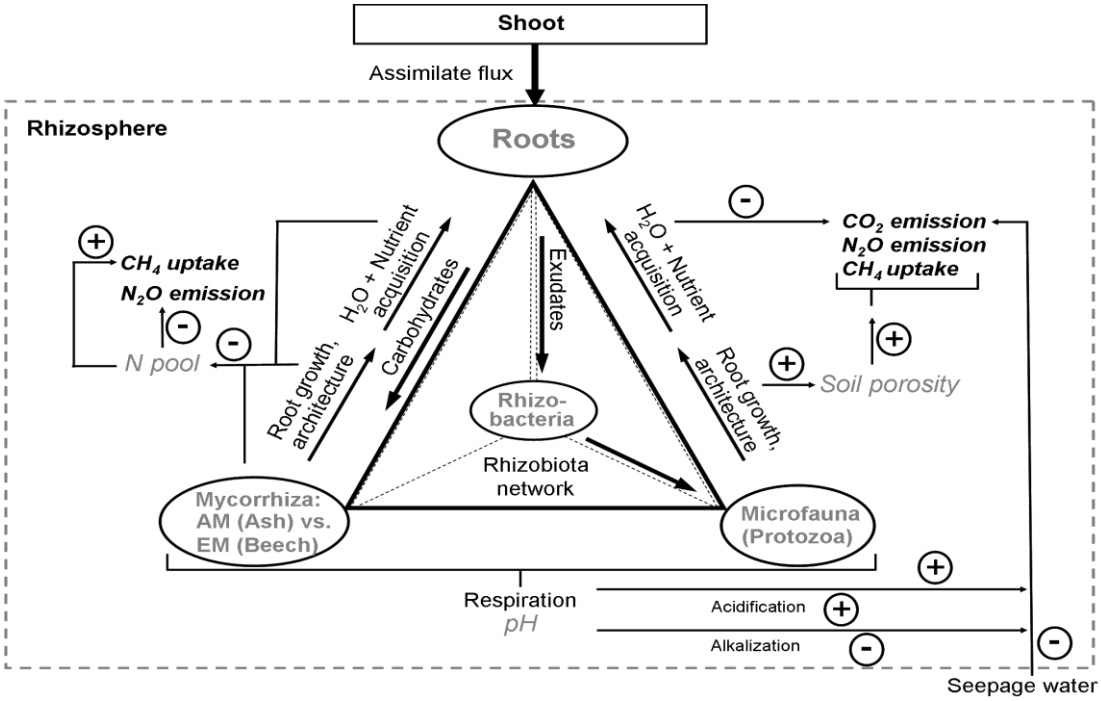

Fig. 5.1. Schematic representation of the biochemical rhizosphere processes and pools involved in the gas exchange of CH_4, CO_2 and N_2O between soil and atmosphere.

More recently, evidence has accumulated that the root systems of tree species growing in mixture may utilise soil resources in a complementary way, i.e. exhaust the available water and/or nutrients to a larger extent than the same species are doing in monoculture (Kelty, 1992; Brassard et al., 2011). So far, no information exists whether tree species mixtures are deviating from the respective monocultures with respect to gas fluxes between soil and atmosphere. Whether tree species effects and root system interactions are relevant forces influencing the source/sink function of forest soils for C and N is best examined in manipulative laboratory studies using soil columns and pot experiments with defined tree species composition and soil conditions.

We introduced a novel double-split-root rhizotron system for studying species effects on the C and N cycling and related gas fluxes in the rhizosphere of tree saplings grown in neighbourhood of either a conspecific (monoculture) or an allospecific sapling (mixture). The aims of this study were to identify species-specific root-induced differences in CH4, N2O, and CO2 fluxes between atmosphere and soil planted with two common Central European broad-leaved tree species, European beech (*Fagus sylvatica* L.) and European ash (*Fraxinus excelsior* L.) under controlled soil conditions, and to examine the evidence for a putative effect of

species interaction (*Fagus* x *Fraxinus*) on the GHG fluxes. We chose two species with largely different morphology, physiology and phylogeny that are co-occurring in several broad-leaved forest communities of Central Europe and are of moderate to high economic importance for forestry (Ellenberg & Leuschner, 2010). The two species represent different families (Fagaceae and Oleaceae), contrast in their successional status (late-successional vs. early-/mid-successional) and differ with respect to root morphology and mycorrhization (beech: thin fine roots with many root tips and ectomycorrhiza vs. ash: thick fine roots with only few root tips and arbuscular mycorrhiza). According to the considerable differences in root morphology, root system size, and mycorrhiza comprising the rhizobiota network, we hypothesised that (1) the N2O emissions are higher from soils under ash due to a higher C and N supply in the soil than under beech, (2) the CO2 efflux from the soil is higher under ash than under beech due to an assumed higher root growth activity, while (3) CH4 uptake is not different between soil planted with ash or beech saplings, and (4) the interaction of beech and ash roots affects the GHG fluxes in a way that is not simply an additive effect of the fluxes in the monocultures. In order to investigate only root-induced effects on GHG fluxes, we excluded the decomposition of leaf litter as a confounding process in our experimental design.

5.3 MATERIALS AND METHODS

5.3.1 Plant and soil material

Plant and soil material was collected in a temperate mixed broad-leaved forest in western Thuringia, Germany, the Hainich National Park (51°04' N 10°30' E, about 350 m a.s.l). *F. fagus sylvatica* and *F. excelsior* form species-rich mixed stands together with other broad-leaved tree species of the genera *Tilia*, *Acer* and *Carpinus* (Leuschner et al., 2009). The stands are 27 – 32 m tall and about 80 – 120 years old. Both target species show a vital rejuvenation in the stands. The climate has a sub-continental character with a mean annual temperature of 7.5 °C, and a mean annual precipitation of 590 mm (Deutscher Wetterdienst, 2005). The soil type is a Stagnic Luvisol (IUSS Working Group WRB, 2007). The mineral soil is composed of 1.8% sand, 80.2% silt, and 18.1% clay.

The rhizotrons were filled with mineral soil material taken from the upper 10 cm of the profile in the Hainich forest right below the organic layer. After sampling, the fresh soil was homogenised and coarse-grained soil particles and roots were removed by sieving with a mesh size of 10 mm, preserving most of the mesofauna. The chemical properties of the soil at the start of the experiment are given in Table 5.1. The saplings were excavated close to the soil sampling site in spring 2009. Immediately before the onset of the experiment, the saplings were carefully dug out with intact soil cores to extract the complete root system with only minimal damage. Adherent coarse soil particles were carefully removed from the roots. In order to allow for a complete infection of the roots by the local mycorrhiza community, the sapling root systems and the surrounding soil material were stored together in watered pots overnight. The saplings of beech and ash had an initial shoot height of 23.1 ± 1.2 cm and 17.9 ± 1.1 cm (means ± 1 SE), and a mean tap root length of 12.1 ± 0.7 cm, and 15.4 ± 1.2 cm, respectively. At the beginning of the experiment, the beech and ash saplings had 2 – 5 leaf buds, but no unfolded leaves. The initial root biomass of the two species is given in Table 5.2. Initial aboveground biomass was 1.26 ± 0.27 g for beech and 1.25 ± 0.15 g for ash and it increased to 2.66 ± 0.60 and 3.24 ± 0.59 g, respectively until harvest after 475 d of cultivation. During winter, the beech and ash saplings were leafless for 50 and 110 d, respectively, which is considerably shorter than in nature, especially in the case of beech.

Table 5.1. Chemical properties of the uppermost 20 cm of the soil in the rhizotrons at the start of the experiment and after 475 d of growth of either two beech, two ash, or one beech and one ash sapling (means ± 1 SE; n = 4).

	Start of the experiment	End of the experiment			
		Control	Beech	Ash	Mixed
Bulk density [g cm^{-3}]	n. d.	1.19 ± 0.10	1.03 ± 0.12	1.05 ± 0.04	0.94 ± 0.05
pH (H_2O)	4.56 ± 0.03	4.68 ± 0.10	4.40 ± 0.14	4.75 ± 0.06	4.54 ± 0.15
CEC [µmolc g^{-1} dw]	191.7 ± 11.8	190.7 ± 15.7	182.3 ± 6.1	184.4 ± 8.7	198.2 ± 16.7
Base saturation [%]	22.9 ± 1.3	17.5 ± 1.8	17.8 ± 1.1	20.1 ± 0.5	21.3 ± 1.1
NO_3^- [mg N kg^{-1} dw]	6.39 ± 0.28	42.1* ± 10.8	34.9* ± 3.9	39.0* ± 2.6	33.9* ± 6.6
NH_4^+ [mg N kg^{-1} dw]	7.85 ± 0.28	4.42 ± 1.58	2.20* ± 0.80	1.66* ± 0.57	1.56* ± 0.50
C_{org} [g kg^{-1} dw]	19.2 ± 0.3	18.3 ± 0.5	17.5* ± 0.5	17.0* ± 0.4	16.4* ± 0.5
N_{total} [g kg^{-1} dw]	1.64 ± 0.01	1.68* ± 0.07	1.63 ± 0.04	1.56* ± 0.02	1.54* ± 0.06
C:N ratio [g g^{-1}]	11.7 ± 0.14	10.9 ± 0.3	10.8 ± 0.2	10.9 ± 0.2	10.6 ± 0.1

None of the nine parameters differed significantly ($P < 0.05$) between the four treatments at the end of the experiment (Tukey-Kramer test); significant differences between a treatment's final state and the conditions at the start of the experiment are indicated by * ($P < 0.05$, paired t test); n.d. = not determined.

5.3.2 Experimental setup

The novel double-split-root rhizotrons are made of anodised aluminium plates with a transparent 10 mm acrylic glass front to observe root growth and death. The total volume of a rhizotron is 15.2 L (600 mm x 900 mm x 30 mm, w x h x d, Fig. 5.2), which is divided by two vertical bars in three compartments at a volume ratio of 1:2:1. The rhizotrons can be thermally regulated by a pipe system of circulating water installed in the back plate and driven by a water pump (Master DW 5500e, Sicce S.p.A., Pozzoleone, Italy). Thereby, thermal homogeneity can be maintained in the entire soil volume of each rhizotron as well as among all 16 rhizotrons used in the experiment. The rhizotrons are equipped with 24 raster access ports (RAP) located in six soil depths to allow for minimal-invasive sampling of bulk soil and rhizosphere solution via steel capillaries as described by Blossfeld et al. (2011). Each RAP field consists of a raster plate of 34 x 34 multiple-step holes (d = 0.7 / 0.5 mm, distance 1 mm) for inserting steel capillaries at defined depths which serve as microsuction cups. By rotation and perpendicular movement of a RAP between the

Table 5.2. Fine, coarse and total root biomass at the beginning and end of the experiment (day 475) in rhizotrons planted either with two beech, two ash or one beech with one ash sapling. Given are means ± 1 SE (n = 4).

	Start of the experiment					End of the experiment				
	Beech	**Ash**	**Mixed (total)**	**Mixed (beech)**	**Mixed (ash)**	**Beech**	**Ash**	**Mixed (total)**	**Mixed (beech)**	**Mixed (ash)**
Root $mass_{fine}$	0.33 ± 0.10	0.81 ± 0.16	0.46 ± 0.09	0.16 ± 0.05	0.41 ± 0.08	1.56^{a} ± 0.54	4.76^{b*} ± 0.91	4.07^{ab} ± 0.76	1.80^{*} ± 0.37	2.27 ± 0.63
Root $mass_{coarse}$	1.28 ± 0.33	1.12 ± 0.12	1.20 ± 0.15	0.64 ± 0.17	0.56 ± 0.06	3.79^{aA} ± 1.07	11.93^{b*} ± 2.94	13.45^{b} ± 2.23	5.24^{B*} ± 1.16	8.21± 2.70
Root $mass_{total}$	1.61 ± 0.42	1.94 ± 0.16	1.77 ± 0.18	0.80 ± 0.21	0.97 ± 0.08	5.34^{aA} ± 1.54	16.69^{b*} ± 3.65	17.52^{b} ± 2.59	7.04^{B*} ± 1.53	10.47 ± 3.04

Different lower case letters indicate significant differences between treatments ($P < 0.05$, Tukey-Kramer test), upper case letters signed differences for a species between monospecific and mixed rhizotrons ($P < 0.05$, Tukey-Kramer test); significant differences between start and end of the experiment are indicated by an asterisk after the values at the end of the experiment ($P < 0.05$, paired t test).

front and back plate, the microcapillary raster fields can be positioned close to the surface of target roots (distance 0.5 mm) in order to allow sampling of rhizosphere solution at variable distances to the root surface and along the root axis. The RAPs are equipped with sterile filter membranes (cyclopore track-etched membranes of 47 mm diameter and a pore width of 0.2 µm, Whatman, Piscataway, NJ, USA) to guarantee for sterile sampling of rhizosphere and soil solution. Opposite to each RAP, observation and manipulation windows (d = 52 mm) made of acrylic glass of reduced thickness (1 mm) are installed in the front plate. The rhizotrons are tilted by 35° in forward direction to induce root growth along the transparent front plate. During the experiment, the front plates were kept covered with black scrim to exclude light penetration to the soil which could have influenced root growth and soil fauna activity.

A one-factorial fully randomised experiment with two blocks and sixteen double-split-root rhizotrons was set up with species composition being varied (ash, beech, mixed). Each eight rhizotrons were installed in a climate chamber (blocks) of the Experimental Botanical Garden of the University of Göttingen with a controlled temperature regime, relative air humidity, and light supply. The experiment consisted of four treatments, each replicated four times: mono-specific beech rhizotrons planted with two beech saplings, mono-specific ash rhizotrons planted with two ash saplings, mixed rhizotrons planted with one beech and one ash sapling, and an unplanted (root-free) control (bare soil).

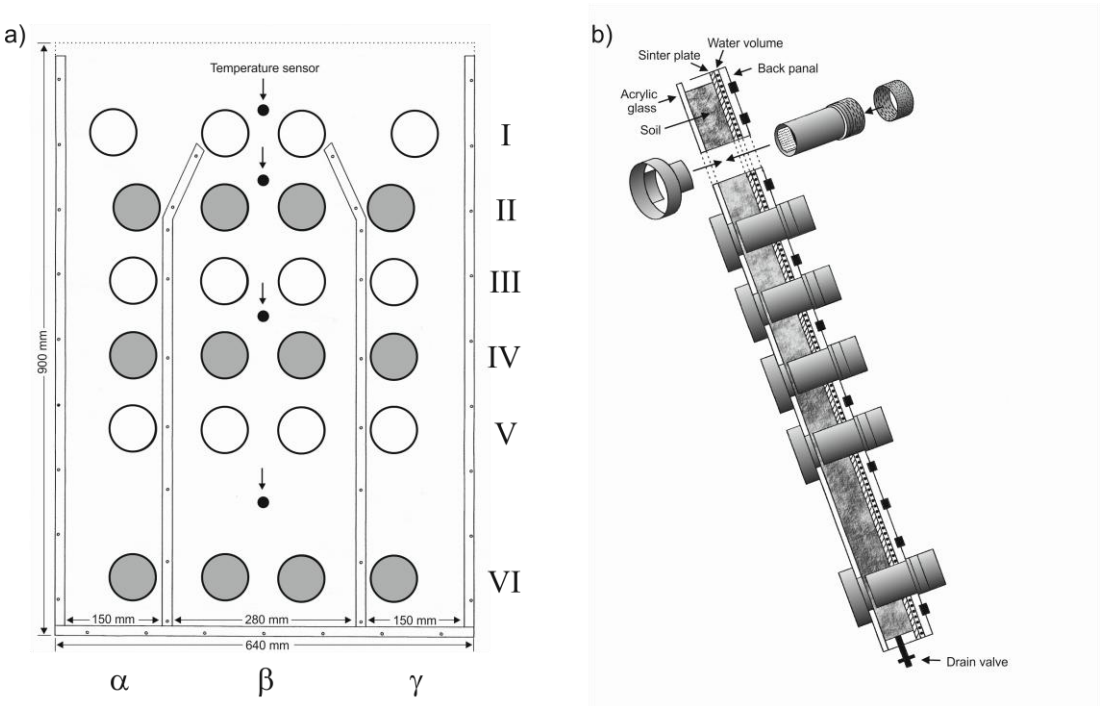

Fig. 5.2. a) Front view of a double-split-root rhizotron. Two metal bars separate the soil volume into three compartments (α, β, γ) at a ratio of 1:2:1. Roman numerals mark the six soil layers in the rhizotron that were accessible by each four cylindrical openings for inserting litter material as a stimulant of local soil biological activity. Black dots mark the position of temperature sensors. The circles mark the position of the raster access ports (upper surface of rhizotron) and the corresponding openings for material addition at the lower surface that could also be used for direct observation through the acryl glass window. In the shaded circles, 1.5 g of ash litter were added to stimulate soil biological activity; no litter was added in the clear circles. b) Longitudinal view of a rhizotron. The soil layer in the rhizotron has a width of 30 mm. For the uppermost soil layer, the design of a raster access port (upper side) and the front ring of 1 mm thick acrylic glass of the observation window (lower side) are shown in detail. The black squares symbolise the position of the water circulation system for thermal regulation of the soil.

All rhizotrons were homogeneously filled with 15.2 L of fresh, sieved soil material (gravimetric water content: 22.7%) before planting. Each of the two saplings per rhizotron was planted right above one separating aluminium bar in the boxes which created three soil compartments (α, β, γ compartment); thus, the roots of the two saplings had a free choice of growing into the three soil compartments (Fig. 5.2a). For simulating the patchy nutrient distribution which is typical for many forest soils, we created local well-defined hot spots of nutrient availability in the soil volume of the rhizotrons by adding a total of 18 g ash leaf litter to every rhizotron on August 22, 2009, i.e. 53 d after planting. This was done by inserting each 1.5 g of litter, diluted by 38.5 g of soil material, to 12 systematically distributed patches of the rhizotron that were accessible by the observation and manipulation windows opposite to the RAPs (layer I: at 0 – 192 mm soil depth, without litter; layer II: 192 – 305 mm depth with litter, layer III: 305 – 417 mm depth without litter; layer IV: 417

– 530 mm depth with litter; layer V: 530 – 698 mm depth without litter; layer VI: 698 – 900 mm depth with litter, Fig. 5.2).

The experiment was conducted under constant climate conditions (20 °C air temperature, 70% relative air humidity) and 10 to 14 h daylight with 203 ± 10 µmol m^{-2} s^{-1} PPFD (EYE Lighting, Clean Ace, Mentor, OH, USA) from June, 30, 2009 until October, 18, 2010 (475 d). The volumetric soil water content was monitored three times a week with a mobile TDR probe (Trime-FM, IMKO, Ettlingen, Germany), and kept at constant level by adding distilled water if a deviation from the target value (21 vol.-%) was observed. Soil temperature was measured with four NTC thermistors per rhizotron (Epcos, Munich, Germany), positioned vertically in the centre of the rhizotrons (compartment β) at soil depths of 80, 200, 425, and 705 mm with 20 mm distance to the acrylic glass front plate. Data were recorded in 15 min-intervals with a CR1000 data logger (combined with two AM416 Relay Multiplexers, Campbell Scientific Inc., Utah, USA).

5.3.3 Measurement and analysis of gas fluxes

The rhizotrons were established three months before starting gas flux measurements in order to adjust the soil to the experimental conditions and to balance the gas exchange after disturbing the natural soil structure. Gas fluxes were measured biweekly at the soil surface for a period of 324 d until harvest applying the closed-chamber technique. A chamber was positioned between the two saplings of each rhizotron (soil surface of compartement β). To create a sufficiently large headspace volume of 1.75 L we used brass chambers with dimensions of 350 mm x 170 mm x 29.5 mm (h x w x d). During the gas flux measurements, the chambers were closed for 1 h. After 0, 20, 40 and 60 min, gas samples were taken from the chamber headspace by flushing gas-tight 50 mL-sample syringes with headspace air, using a cannula and two three-way valves. The gas concentrations were analysed by a gas chromatographic system. A detailed description of the GC configuration is presented in Loftfield et al. (1997). The fluxes were calculated from the linear concentration change during the time of chamber closure.

5.3.4 Sampling of rhizosphere solution and analysis of organic acids by capillary electrophoresis-UV

Before final harvest, rhizosphere solution was collected to identify organic acids originating from root exudates of beech or ash saplings. Each four transverse transects to beech and ash fine roots were investigated at distances of 1, 6 and 11 mm to the root surface. The roots used for this investigation all grew within the upper 650 mm of the soil column. For comparison, soil solution was also extracted at three randomly selected locations in the soil of the (root-free) control rhizotrons. In order to generate sufficient solution for sampling, the soil was irrigated with 1.67 L m^{-2} of distilled water 1 h before sampling. Capillaries of stainless steel (d_o = 0.6 mm, d_i = 0.4 mm, length = 1000 mm, SWS Edelstahl GmbH, Emmingen, Germany) were inserted into the RAPs. Before sampling rhizosphere solution, the system was flooded with 70% ethanol to sterilise it. To avoid a blockage of the capillaries by particles, a steel wire (d = 0.3 mm, wiped with 70% ethanol) was inserted into each capillary during the process of positioning into the raster plates. The wire was removed before collecting the rhizosphere solution. The capillaries were connected to a vacuum sampling chamber (Blossfeld et al. 2011). The pressure in this chamber was lowered by 320 hPa to a level of 650 hPa with a diaphragm pump (Typ MZ 2, Vacuubrand GmbH, Wertheim/Main, Germany). We collected soil solution volumes in the range from a few µL to 1.5 mL in Eppendorf caps of 1.5 mL volume over 60 min.

All samples were analysed for organic acids (oxalate, formate, acetate, lactate) using capillary electrophoresis with a salicylate electrolyte (Bazzanella et al., 1997). A capillary electrophoresis system G1600A (Agilent, Böblingen, Germany) was used, equipped with a built-in diode-array detector. Fused silica capillaries (Polymicro, Phoenix, USA) of 75 µm I.D. x 64.5 cm total length (56 cm to detector) were used. The electrolyte solution contained 7.5 m_M salicylic acid, 15 m_M TRIS, 0.5 m_M dodecyltrimethylammonium hydroxide and 0.3 m_M $Ca(OH)_2$. A voltage of 30 kV was applied during all separations, with temperature maintained at 25 °C. Injections were carried out hydrodynamically with a pressure of 50 mbar for 30 s. The separated compounds were detected by indirect UV-detection at a wavelength of 232 nm. Quantification was performed using external calibration with aqueous standard

solutions (5, 10, 20 µ*M*) and internal standardisation using phenylacetic acid as internal standard. The rhizosphere samples (12.5 µL/ash, 10 µL/beech) were diluted with deionized water (77.5 µL/ash, 80 µL/beech) and 10 µL of a 100 µ*M* internal standard solution prior to analysis. Calibration lines for the organic acids investigated were linear in the range between 5 and 20 µ*M* with correlation coefficients from 0.9965 to 0.9991. The limits of detection (LOD, 3σ) were about 0.5 – 1.0 µ*M*.

5.3.5 Plant harvesting and soil analysis

At the first day of harvest (475 d after planting), the shoot length and root collar diameter of each sapling were measured. The roots were carefully excavated from the soil, washed and cleaned from adherent soil particles. Where possible, three representative root branches per species and soil compartment were isolated in all six soil layers of the rhizotrons and digitalised on a flat-bed scanner for image analysis to determine specific fine root area (SRA, cm^2 g-1 dry matter), specific fine root length (SRL, cm g^{-1} dry matter) and total fine root surface area using WinRhizo 2005c software (Régent Instruments Inc., Québec, QC, Canada). All biomass samples were oven-dried (70 °C, 48 h) and weighed for dry weight determination. For quantifying the vertical distribution of root biomass in the rhizotrons, we calculated the relative cumulative root biomass in the six soil depth layers of the boxes and described the depth distribution by the exponential function $y = 1 - \beta^d$ given by Gale and Grigal (1987) which expresses the cumulative proportion of root biomass y as a function of soil depth d and a specific factor β. The relative growth rate of the roots (RGR, mg d^{-1} g-1 root mass) was estimated by subtracting the initial root mass (determined in five saplings per species at the day of planting) from the root mass of the harvested saplings divided by the duration of the experiment and relating to initial root mass. The plant material was grounded with a disc mill and the C and N concentrations detected in a mass spectrometer (Delta plus, Finnigan MAT, Bremen, Germany). The colonisation with AM and EM was determined as described previously (Lang et al., 2011a), and differed between ash (85%) and beech (44%), but not between mono or mixed systems. Compared to field observations, both ectomycorrhizal and arbuscular mycorrhizal colonisation rates were within typical ranges (Pena et al., 2010; Lang et al., 2011a).

For estimating the root-induced respiratory activity in immediate vicinity of the roots, we estimated root respiration by calculating root growth respiration from the expression $R_g = (RGR_{root}+73.7)/4.31$ given by Reich et al. (1998) with RGR_{root} being the relative growth rate of the roots, and on the assumption that root maintenance respiration is approximated by $R_m = 0.106 \times N$ following Ryan (1991) with N being the nitrogen concentration of root dry mass. By subtracting the calculated root respiration (growth plus maintenance respiration) and the pure soil respiration, measured in the root-free rhizotrons, from the measured total net CO_2 efflux, we obtained an estimate of the root-induced additional soil respiration in the rhizosphere. The root respiration rates of ash calculated from root RGR and root N content were checked against independent *in situ* measurements conducted with miniature root cuvettes (1.5 mL microcentrifuge tubes) placed around root segments of ash saplings (n = 5) using miniature planar CO_2 optodes (Presens, Regensburg, Germany) for online optoanalytical measurement of net CO_2 release over 180 min at one minute intervals..

During the harvest soil samples from the upper 20 cm-layer located below the gas flux sampling area were extracted for chemical analysis. To exclude an effect of soil depth on soil properties additional samples were taken in each soil layer. The soil pH was analysed in a suspension with 10 g soil and mixed with 25 mL H_2O using a Vario pH meter (WTW GmbH, Weilheim, Germany). The gravimetric soil water content was determined by weighing the soil samples before and after drying at 105 °C for 24 h. The nitrate (mg $N\text{-}NO_3^-$ kg^{-1} dw) and ammonium (mg $N\text{-}NH_4^+$ kg^{-1}dw) concentrations were estimated by extracting soil samples in 0.5 M K_2SO_4 solution (1:3 wet soil mass to solution ratio) directly after collection. The samples were shaken for 1 h and passed through folded filters (150 mm in diameter, 65 g m^{-2}, Sartorius Stedim, Aubagne, France). The NO_3^- and NH_4^+ concentrations of the filtered extracts were analysed using continuous flow injection colorimetry (SAN^+ Continuous Flow Analyzer, Skalar Instruments, Breda, The Netherlands). Nitrate was determined by the copper cadmium reduction method (ISO method 13395) and NH_4^+ by the Berthelot reaction method (ISO method 11732). The contents of C_{org} and N_{total} were determined in a mass spectrometer (Delta plus, Finnigan MAT, Bremen, Germany) after grounding the dry soil in a disc mill. The bulk density of the

material was determined in 5 cm soil depth under the gas flux sampling area using plastic cores with a defined volume of 10.8 cm^3 after Schlichting et al. (1995). The particle size distribution of the soil material in the rhizotrons was analysed in five replicate samples using the sieving and pipette method (Schlichting et al., 1995).

5.3.6 Data analysis

All statistical analyses were carried out with SAS 9.1 software (Statistical Analysis System, SAS Institute Inc., Cary, NC, USA). Cumulative gas fluxes were calculated by summing up all measurements done in a rhizotron considering the number of measurements taken and the length of the entire measuring period (324 d). The gas fluxes varied considerably between the different measurement days as it is common for GHG fluxes from soil, so that we refrained from showing the time course. All data were tested for normal distribution using the Shapiro-Wilk test and for homogeneity of variances applying the Levene test. The block effect of the two climate chambers was tested with a two-factorial ANOVA considering the two factors "treatment" and "bloc" and an interaction term ("treatment x block"). For the various soil chemical properties, the gas fluxes and the biological parameters, no bloc (chamber) effect was detected. To investigate the effects of beech and ash roots on various parameters, one-way ANOVA with a post hoc Tukey-Kramer test was used to locate significant differences among treatment means for data showing normal distribution. If the data were not normally distributed or variances were not homogeneous, the non-parametric Kruskal-Wallis test was used to test for significant differences between means. The significance of differences between two treatments was subsequently investigated with the Wilcoxon U-test. A paired t test was used to test for significant differences in normally distributed soil parameters between the soil state at harvest and the experiment's beginning. Linear regression analysis was conducted to relate the chemical soil properties of the uppermost 20 cm to various biological parameters (listed in Tables 5.1 and 5.2), and to relate gas fluxes to soil chemical properties and biological parameters. In all analyses, significance was determined at $P < 0.05$.

5.4 RESULTS

5.4.1 Gas fluxes

The uptake of CH_4 by the soil was found to be 173 % higher in ash rhizotrons than in the root-free control rhizotrons with significant differences to both the control and to the mono-specific beech rhizotrons (Fig. 5.3a). Beech and control rhizotrons showed comparable CH_4 fluxes (-46.8 ± 5.0 and -38.5 ± 9.1 mg C m^{-2} 324 d^{-1}). In the mixed rhizotrons, intermediate uptake rates were measured. We found a close negative correlation between CH_4 uptake rate and the extractable NH_4^+ concentration in the soil across the four treatments (R^2 = 0.33; P = 0.020, Fig. 5.4a) while the CH_4 fluxes were positively related to fine root biomass (R^2 = 0.38; P = 0.032, Fig. 5.4b).

The cumulative N_2O emission over the experimental period was approximately 30% smaller from the rhizotrons with ash saplings (0.40 ± 0.09 g N m^{-2} 324 d^{-1}) than from the other three treatments (means of 0.55, 0.63 and 0.60 g N m^{-2} 324 d^{-1} in the mixed, mono-specific beech and root-free control rhizotrons, respectively, Fig. 5.3b).

However, the variation in N_2O fluxes among the replicate rhizotrons (n = 4) was large with values ranging from 0.15 to 1.03 g N m^{-2} 324 d^{-1} in the control. As a consequence, the difference between the ash and the beech rhizotrons was only marginally significant (P = 0.056). We found a highly significant negative relation between the cumulative N_2O emission and the salt-extractable NO_3^- concentration in the soil across the four treatments (R^2 = 0.74, P < 0.0001, Fig. 5.4c). A negative

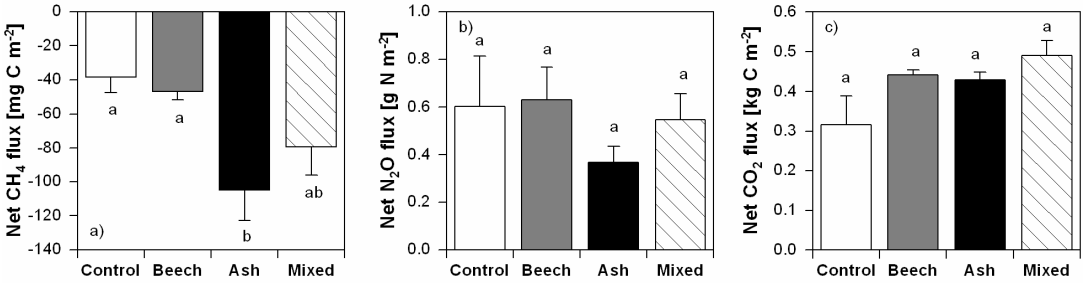

Fig. 5.3. Fluxes of CH_4, CO_2 and N_2O measured at the soil surface of rhizotrons planted with beech and/or ash saplings, cumulated for 324 d (biweekly measurements). Given are data for rhizotrons planted with no saplings (bare soil, control), or rhizotrons with two beech (beech), two ash (ash), or one beech and one ash sapling (mixed); n = 4; means ± 1 SE. Different lower case letters indicate significant differences between the treatments (for CH_4: Tukey-Kramer test; for N_2O and CO_2: Wilcoxon U-test, each with P < 0.05).

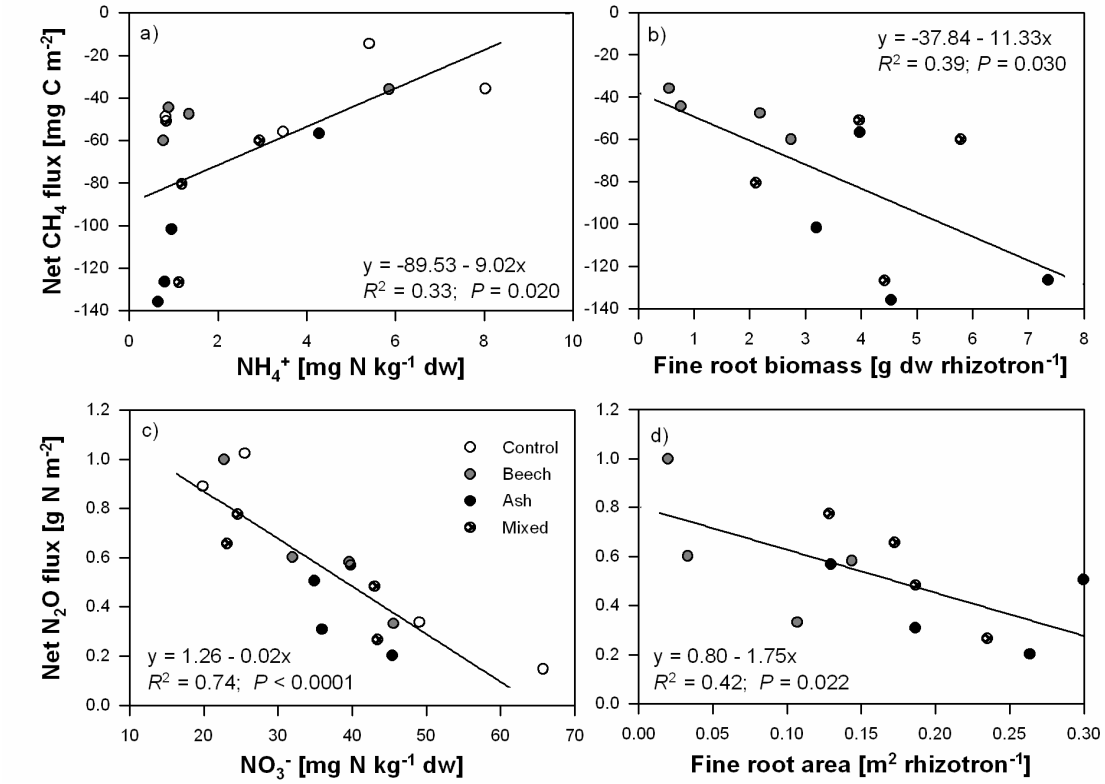

Fig. 5.4. Relationships between cumulative N_2O fluxes (period: 324 d) in rhizotrons planted either with beech or ash saplings or a beech/ash mixture and (a) the NH_4^+ concentration in the uppermost 200 mm of the soil or (b) total fine root biomass in a rhizotron. Relationships between the cumulative CH_4 uptake of the soil (period: 324 d) and (c) the NO_3^- concentration in the uppermost 20 cm of the soil or (d) the total fine root surface area in a rhizotron; n = 4 rhizotrons per treatment in all cases.

correlation also existed between the N_2O flux and total fine root area in a rhizotron (R^2 = 0.42; P = 0.020, Fig. 5.4d).

The cumulative net release of CO_2 from the soil tended to be higher by 40% and 36% in the mono-specific beech and mono-specific ash rhizotrons than in the control rhizotrons, but the difference was not significant (Fig. 5.3c). The highest CO_2 emission was measured in the mixed rhizotrons that contained beech and ash roots (0.55 ± 0.04 kg C m^{-2} 324 d^{-1}); the differences to the root-free control (55% higher) were marginally significant (P = 0.056). Mass-specific root respiration as calculated from relative root growth rate and root N concentration was higher in the mono-specific ash rhizotrons (26.7 ± 0.6 nmol CO_2 g dw^{-1} s^{-1}) than in the beech rhizotrons (22.0 ± 0.6 nmol CO_2 g dw^{-1} s^{-1}). A similar species ranking was found for root respiration in the mixed rhizotrons (27.9 ± 1.2 nmol CO_2 g dw^{-1} s^{-1} for ash and 24.8 ± 0.9 nmol g dw^{-1} s^{-1} for beech; Table 5.3). The net CO_2 release was not related to any of the chemical properties of the topsoil or root morphological parameters listed in Table 5.1 (data not shown).

Table 5.3. Partitioning of measured net CO_2 efflux from the soil (cumulated over 324 d from biweekly measurements) into the components soil respiration (respiration of root-free control), root respiration and root-induced soil respiration (in kg C m^{-2} 324 d^{-1}: left columns, or % of total respiration: right columns; n = 4 rhizotrons).

	Control		Beech		Ash		Mixed (total)	
Total respiration	$0.317^{a} \pm 0.072$	100	$0.442^{a} \pm 0.013$	100	$0.429^{a} \pm 0.020$	100	$0.490^{a} \pm 0.038$	100
Soil respiration	$0.317^{ab} \pm 0.072$	100	$0.317^{a} \pm 0.072$	71.9 ± 2.0	$0.264^{b} \pm 0.030$	61.6 ± 6.8	$0.271^{ab} \pm 0.030$	54.9 ± 3.5
Root respiration	-	-	$0.040^{a} \pm 0.012$	9.0 ± 2.7	$0.152^{b} \pm 0.036$	35.5 ± 8.5	$0.162^{b} \pm 0.029$	34.8 ± 8.3
Root-induced soil respiration	-	-	$0.085^{a} \pm 0.010$	19.1 ± 2.0	$0.013^{b} \pm 0.013$	2.9 ± 2.9	$0.058^{ab} \pm 0.039$	10.2 ± 6.9

Root respiration was estimated from relative root growth rate (growth respiration) and root N concentration (root maintenance respiration); see text. Root-induced soil respiration was calculated as the difference between net CO_2 efflux and soil plus root respiration. Lower case letters show significant differences between the respiration rates of each treatment (Wilcoxon U-test, $P < 0.05$).

5.4.2 Organic acids in the rhizosphere solution

According to the micro-capillary extraction at 1 – 11 mm distance to the root surface, the average concentration of organic acids (oxalate, formate, acetate and lactate) tended to be higher in the vicinity of beech roots than close to ash roots (432 ± 103 µ*M* vs. 314 ± 101 µ*M*). The analysis revealed higher concentrations of acetate in direct contact (1 mm distance) to ash roots as compared to beech roots. In the rhizosphere of ash roots, the acetate concentration rapidly decreased with root distance from > 70 µ*M* at 1 mm to 30 µ*M* at 11 mm (Fig. 5.5). Both oxalate and formate decreased (from 20 µ*M* to 7 µ*M* and from 47 µ*M* to 20 µ*M*, respectively) with increasing distance from ash roots while the concentrations of oxalate and formate remained more or less invariant between 1 and 11 mm distance to beech roots. Formate was the most abundant organic acid found near beech roots (53 – 87 µ*M*). The qualitative differences in the composition of the soil solutions near beech and ash roots are illustrated by the fact that the acetate concentration contributed by 47% to the total concentration of organic acids in the vicinity of ash roots, while 45% of the organic acids were formate near beech roots. In root-free bulk soil, the soil solution contained exclusively oxalate and formate (32 ± 21 and 45 ± 15 µ*M*, respectively) while acetate and lactate was solely detected in the vicinity of roots (Fig. 5.5).

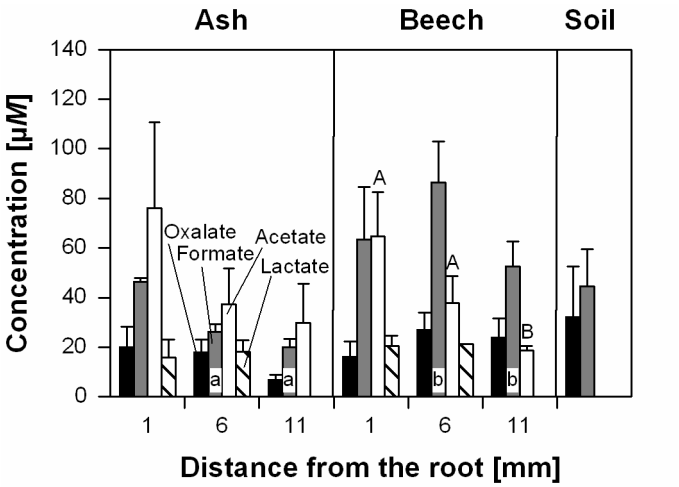

Fig. 5.5. Variation in organic acid concentration (oxalate, formate, acetate and lactate) in the rhizosphere solution at three distances from the root surface, measured in orthogonal direction from fine root branches of beech or ash saplings (n = 4, means ± 1 SE) and control measurements in root-free rhizotrons (n = 3). Significant differences between beech and ash for the corresponding distances were marked with lower case letters, differences within a transect marked by upper case letters (Wilcoxon U-test, $P < 0.05$) To keep the figure readable we omitted letters indicating non- significant differences. For the lactate concentration in 6 mm distance from the beech root, no SE could be calculated due to values below the detection limit.

5.4.3 Root biomass and soil properties

Ash saplings produced about three times as much root biomass (fine, coarse and total) during the experimental period of 475 d than beech saplings (Table 5.2). In the mixed rhizotrons, root biomass was similarly large as in the mono-specific ash rhizotrons showing no evidence of belowground 'overyielding' in terms of standing root mass. However, the beech saplings grown in mixture produced on average significantly more fine and coarse root biomass than beech saplings planted in mono-specific culture, while ash root biomass was not significantly affected by the neighbour identity (Table 5.2). Most of beech root biomass, i.e. 82% to 98%, was located in the upper 30 cm of the rhizotrons, whereas the corresponding relative proportion of ash roots was 59 to 91%, evidencing a more deep-reaching soil exploration in ash (Fig. 5.6). None of the investigated soil chemical and physical parameters of Table 5.1 showed a significant relation to the fine or total root biomass of the two species in the rhizotrons (data not shown).

At the end of the experiment, the chemical properties of the soil in the rhizotrons differed not significantly between the four treatments (Table 5.1). However, C_{org} decreased in the rhizotrons by 0.9 g C kg^{-1} to 2.8 g kg^{-1} dw during the experimental period and the decrease tended to be largest in the treatment with beech/ash mixture. The reduction led to C concentrations that were by 10% lower than in the control

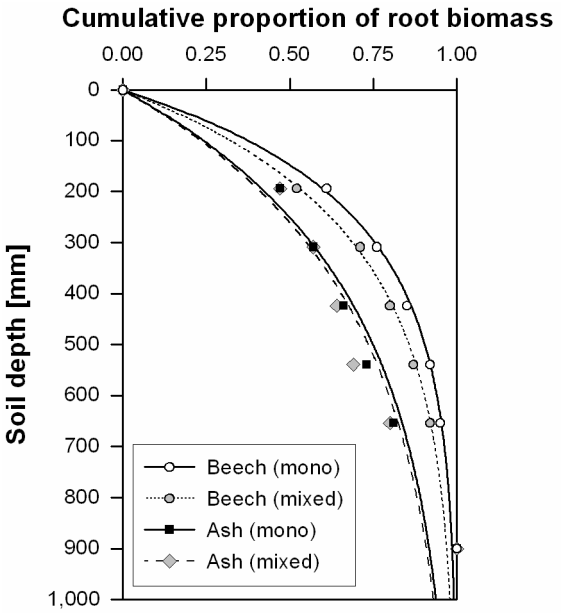

Fig. 5.6. Cumulative amount of root biomass as a function of soil depth in the rhizotrons planted either with two beech, two ash, or one beech and one ash sapling (relative units, means of n = 4 rhizotrons). Each seven measurements were conducted per rhizotron along the profile. The shape of the curve is described by the β-value of the regression equation $y = 1 - \beta^d$ after Gale and Grigal (1987, see text). Mono-specific beech rhizotrons: $\beta = 0.954$, $R^2 = 0.999$, $P < 0.001$; mono-specific ash rhizotrons: $\beta = 0.973$, $R^2 = 0.978$, $P < 0.001$; beech in mixed rhizotrons: $\beta = 0.962$, $R^2 = 0.998$, $P < 0.001$; ash in mixed rhizotrons: $\beta = 0.974$, $R^2 = 0.966$, $P < 0.001$.

rhizotrons where plants were absent ($P = 0.056$). The pH (H_2O) value tended to be 0.4 units higher under ash than under beech saplings ($P = 0.056$) indicating that root-induced acidification was more pronounced by beech than by ash. Large changes occurred over the experimental period in the soil content of salt-extractable inorganic nitrogen: the NH_4^+ concentration decreased and the NO_3^- concentration increased in the 475 d period; these changes were influenced by the presence of the tree saplings. At the end of the experiment, the rhizotrons with saplings contained by 50% to 65% smaller NH_4^+ concentrations than the control soil. The NO_3^- concentration increased by 399% to 526% during the experiment with a relatively small increase found in the mixed rhizotrons and a large increase in the control (Table 5.1). No changes in soil properties with soil depth were observed. Regression analyses showed that root-related parameters (total fine root area, fine root biomass per rhizotron) had no influence on the extractable NO_3^- and NH_4^+ concentrations in the soil ($P > 0.05$).

5.5 DISCUSSION

While field studies in mature forests provide valuable information on the average source or sink strength of the soils and seasonal flux dynamics, it is more difficult to understand the controlling factors of CH_4 uptake and N_2O release in different forest

types because differences in tree species composition are typically associated with differences in soil physical and chemical conditions which complicates the separation of tree species from soil effects on GHG exchange (Fig. 5.1). By excluding litter fall and controlling for temperature, soil moisture, soil bulk density and initial soil N and C content, our investigation focused on possible root-induced effects of different tree species on GHG fluxes in order to disentangle biotic and abiotic controls of N_2O emission and CH_4 uptake in temperate mixed forests.

5.5.1 CH_4 uptake

The recorded CH_4 uptake rates in the rhizotrons (0 – 40 µg C m^{-2} h^{-1}) showed a similar magnitude as CH_4 fluxes measured under field conditions in the Hainich forest (0 – 78 µg C m^{-2} h^{-1}, Guckland et al., 2009). The specific effect of ash roots was visible in the cumulative rate of CH_4 uptake which was by 173% higher in rhizotrons planted with ash saplings than in the control soil, and by 124% larger than in beech rhizotrons. Thus, the observed stimulation of CH_4 oxidation in our experiment was mainly a consequence of the presence of ash roots, while beech roots increased the uptake by only 22% (non-significantly) compared to the control. Beside this species effect, we found a close positive relation between CH_4 uptake and the amount of fine root biomass in the rhizotrons across all treatments. It is known that CH_4 uptake is particularly susceptible to variation in soil moisture and gas diffusivity (Smith et al., 2000). Because soil moisture and soil bulk density were similar among the treatments, differences in oxygen supply cannot explain the higher CH_4 oxidation in rhizotrons with tree saplings compared to the root-free control, and in the ash treatment in particular. This conclusion is supported by the occasional measurement of O_2 partial pressure in the bulk soil using O_2-sensitive optodes which showed that the O_2 pressure in the soil (about 200 hPa) was close to atmospheric O_2 partial pressure in all rhizotrons (data not shown, the optodes consisted of a PSt1 sensor with a Microx TX3 device, Presens, Regensburg, Germany).

The oxidation of CH_4 depends on the initial CH_4 concentration in the soil volume (Le Mer & Roger, 2001). In our rhizotrons, the concentrations were at an ambient atmospheric level between 1640 and 1890 ppb in all treatments. For each measurement date, these initial concentrations did not differ among the treatments (tested with Wilcoxon U-test). Methane oxidation is known to be sensitive to NH_4^+

fertilisation either through competitive inhibition of methane monooxygenase by NH_4^+ or through a negative salt effect in fertilisation experiments (Steudler et al., 1989; Bodelier, 2011b). We found a significant negative relation between CH_4 uptake rate and extractable NH_4^+ concentration in our experiment (R^2 = 0.33, P = 0.02), which is hardly explicable by a salt effect on the methanotrophs because the inorganic N concentrations were rather small. Reduced NH_4^+ concentrations in the planted rhizotrons compared to root-free soil (means of 1.56 – 2.20 vs. 4.42 mg NH_4^+-N kg^{-1} dw, respectively) can be one possible explanation for the observed higher CH_4 uptake rates in the treatments with tree saplings than in bare soil.

The particularly high CH_4 uptake rates in the ash treatment might well be a consequence of the deeper reaching root system of this species in comparison to beech which rarely exceeded a maximum rooting depth of 60 cm (0 – 3% of fine root biomass), while 1 – 14% of ash root biomass was located below 60 cm. As has also been observed in field studies (Rust & Savill, 2000; Meinen, Leuschner, et al., 2009), ash saplings showed a higher production of fine and also coarse roots, explored the subsoil in the rhizotrons more rapidly and reached higher densities of fine root mass per soil volume (0.60 ± 0.05 and 0.26 ± 0.10 g L^{-1} in the upper 20 cm of the mono-specific and mixed rhizotrons, respectively) than beech saplings (0.22 ± 0.05 and 0.23 ± 0.05 g L^{-1}, respectively). We assume that deep-reaching roots create channels of higher gas diffusivity that facilitate the downward transfer of CH_4 in soils.

Finally, the composition and concentration of root exudates may also affect CH_4 oxidation through specific promoting or inhibiting effects. Morphological and physiological differences between ectomycorrhiza (*Fagus*) and arbuscular mycorrhiza (*Fraxinus*) on soil chemistry and related effects on gas fluxes might also be important. This deserves further study.

5.5.2 N_2O emission

The N_2O fluxes measured in the rhizotrons were higher than emission rates recorded under field conditions in the soils of the Hainich forest (19 – 124 vs. < 10 µg N m^{-2} h^{-1}, Guckland et al., 2010). This is a common outcome of experiments (Jungkunst et al., 2008) and can be related to the destruction of soil aggregates leading to a higher bio-availability of C and N, continuously favourable soil moisture and higher temperatures in the laboratory than in the field (20 °C vs. 10 – 20 °C). Overall, the

initial N_2O concentrations were between 290 and 510 ppb and did not differ among treatments for each measurement date (tested with Wilcoxon U-test). The cumulative N_2O emissions from rhizotrons planted with ash were on average by 50 – 60% smaller than those from rhizotrons with beech (mono-specific and mixed), and also than from the root-free control, which points at a suppressing effect of ash roots on the release of N_2O from the soil. Since the variation of gas flux among each of the four replicate rhizotrons was large, which is a characteristic outcome of N_2O flux measurements (Jungkunst et al., 2006, 2008), we found only marginally (P = 0.056) or non-significant differences between the four treatments. Therefore, we discuss the likely trends only briefly.

Across all 16 rhizotrons, we found no relation of N_2O flux to the NH_4^+ concentration but a negative one to NO_3^- concentration.. The latter is best explained by a more rapid NO_3^- depletion with higher denitrification rates, which is a main source of the N_2O released (Davidson et al., 2000; Bateman & Baggs, 2005). A negative correlation was also detected between total fine root surface area (and fine root biomass) in the rhizotrons and the cumulative N_2O emission (R^2 = 0.42, P = 0.020 and R^2 = 0.30, P = 0.065 (data not shown), respectively). Ash with a more rapid root and shoot growth rate must have taken up more N than the slower growing beech (Table 5.4), but a trend for a greater depletion of the NO_3^- and N_{total} pools in the soil by ash as compared to slower growing beech was not found. However, in a ^{15}N tracer field experiment, Jacob et al. (unpublished data) found a larger uptake of NH_4^+ and glycin in ash compared to beech, maple, lime and hornbeam. Therefore, it can be suggested that the ash saplings growing in the rhizotrons took up more N as well. The NO_3^- concentration in the soil was not related to root mass and area, and it did not significantly differ between the treatments. Nevertheless, our results indicate that certain broad-leaved tree species can have a substantial influence on the emission of N_2O from forest soils through their root systems. A root-induced influence on the N_2O release can occur independently from a leaf litter effect, and in the absence of significant alterations in pH, total soil N content or soil C:N ratio, which typically characterise soil patches under beech as compared to ash trees in mixed stands (Neirynck et al., 2000; Frédéric M Holzwarth et al., 2011; Langenbruch et al., 2011).

Table 5.4. Nitrogen net accumulation in the root or total biomass of the tree saplings in the mono-specific beech, mono-specific ash and mixed rhizotrons at the end of the experiment (in mg N per rhizotron; means ± 1 SE; n = 4; each two saplings per rhizotron).

	Beech	Ash	Mixed
N accumulation in root biomass	0.067^{a} ± 0.028	0.317^{b} ± 0.053	0.295^{b} ± 0.039
N accumulation in total plant biomass	0.113^{a} ± 0.036	0.473^{b} ± 0.059	0.478^{b} ± 0.060

5.5.3 CO_2 emission

It has been found notoriously difficult to partition the measured net CO_2 efflux from soils to the relevant sources, i.e. autotrophic respiration (root maintenance and growth respiration), the respiration of bacteria, fungi and animals in the soil matrix, and additional microbial respiration in the immediate proximity of roots that is stimulated by root exudation (root-induced respiration). The CO_2 measurements in this study showed that CO_2 efflux from the treatments with tree saplings was by 36 to 55% higher than from root-free soil, which agrees well with empirical data on the relative importance of autotrophic respiration in beech forests in Central Germany (30 – 35% of total soil respiration in the vegetation period or 50% in August, Brumme et al. 2009). We attempted to obtain a rough quantification of root respiration and root-induced respiration in the rhizosphere by calculating theoretical figures of root respiratory activity from established relations between root growth rate and root N concentration and subsequently relating it to the 'background' respiratory activity in root-free soil. The calculated respiration rates for beech roots (22 and 25 nmol CO_2 g dw^{-1} s^{-1} in mono-specific and mixed rhizotrons) are in the range of rates measured *in situ* in the roots of 10-yr-old beech trees in a beech forest using a cuvette technique applied to isolated root branches (16 nmol CO_2 g dw^{-1} s^{-1}, Gansert 1994). For the ash roots in the rhizotrons, we calculated 27 and 28 nmol CO_2 g dw^{-1} s^{-1}, which is somewhat higher than rates determined by *in situ* measurements in the rhizotrons using planar CO_2 optodes (mean of 19 nmol CO_2 g dw^{-1} s^{-1}, n = 5 roots, data not shown). Since fine and coarse root biomass were about three times larger in the ash rhizotrons than in the beech treatment, the CO_2 release from root respiration must have been much larger in the former with more vigorous root growth. However, from the comparable rates of total soil respiration measured in the two treatments over the 324 d-experimental period (cumulative values of 0.44 and 0.43 kg C m^{-2} ground area in the beech and ash rhizotrons), it follows that beech roots must be responsible for a much higher root-induced soil respiration in the

rhizosphere than ash roots, given that the 'background' soil respiration (adopted from the rates measured in root-free soil) was indeed similar in the two treatments as assumed here. According to this calculation, root respiration contributed with a much larger proportion (about 40%) to total CO_2 efflux in ash rhizotrons than in beech rhizotrons (< 10%), whereas soil respiration and root-induced soil respiration must be relatively more important in the latter. The calculated larger root-induced heterotrophic respiration under beech saplings matches with the larger basal respiration (BAS) in beech compared to ash rhizotrons detected by substrate-induced respiration analysis (data not shown). In the proximity of beech roots, we found on average higher concentrations of organic acids. Because they are a growth substrate for many types of soil bacteria (Brimecombe et al., 2007; Walker et al., 2003), the higher concentration of organic acids might translate into a higher microbial activity in the rhizosphere of this species as compared to ash. This assumption is supported by higher rates of root-induced soil respiration in the rhizosphere of beech than ash roots, as they appeared from our investigation of root and soil respiration. It is remarkable that beech roots apparently stimulated the soil biological activity in the rhizosphere much more than ash roots despite a smaller root growth rate and consequently lower density of roots per soil volume. If this finding is of more general validity, it points at large tree species differences in the effect of roots on rhizosphere processes.

5.5.4 Conclusions

The present investigation of root-induced trace gas fluxes using novel double-split-root rhizotrons shows that broad-leaved tree species may substantially alter the source/sink strength of forest soil for greenhouse gases (GHG) via root-related processes. The comparison of beech and ash indicates that tree species identity needs to be considered as controlling factor of GHG fluxes in temperate forests. We found differing effects of beech and ash on CH_4 uptake, thus our results did not support hypothesis (3). Furthermore, the apparent root effects on GHG exchange occurred without marked changes in bulk soil C and N pools, pH and soil moisture conditions, contradicting hypothesis (1). The significant stimulation of CH_4 oxidation by ash roots was positively related to fine root biomass but the apparent reduction of N_2O release by ash was not. This indicates that it is not simply a quantitative root effect

(more roots lead to lower GHG fluxes) but a qualitative root effect on soil biological activity. The CO_2 efflux data show that roots are capable of influencing soil biological activity through species-specific effects on root-induced soil respiration, which was much higher in beech than in ash. Species differences in the composition and concentration of organic acids in close proximity to fine roots seem to support the proposed qualitative effect, but further analyses are needed. Further, our results provide evidence that beech and ash significantly differ in root respiration under identical ambient conditions; the calculated rates were much higher in ash with faster root growth (hypothesis 2). We found no indication of synergistic effects in the allospecific treatment, contradicting hypothesis (4); the fluxes of N_2O, CH_4 and CO_2 in the mixed rhizotrons could all be explained by adding the activities of the two species.

The calculation of the greenhouse gas balance (total sum of CO_2, CH_4, and N_2O fluxes in CO_2-eq) of our soil from a temperate broad-leaved forest planted with beech and ash saplings under controlled climatic conditions revealed a tendency to a more favourable balance in the presence of ash than of beech (5.4 ± 0.2 vs. 5.9 ± 0.2 g CO_2-eq m^{-2} d^{-1}). Clearly, we carried out these measurements under constant climatic conditions without diurnal and annual variation; nevertheless, the calculations indicate that the stimulation of CH_4 uptake and the reduction of N_2O emissions by ash saplings can compensate higher CO_2 emissions due to more vigorous root growth.

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ROOTS FROM BEECH (*FAGUS SYLVATICA* L.) AND ASH (*FRAXINUS EXCELSIOR* L.) DIFFERENTIALLY AFFECT SOIL MICROORGANISMS AND CARBON DYNAMICS

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6.1 ABSTRACT

Knowledge on the influence of living roots on decomposition processes in soil is scarce but is needed to understand carbon dynamics in soil. We investigated the effect of dominant deciduous tree species of the Central European forest vegetation, European beech (*Fagus sylvatica* L.) and European ash (*Fraxinus excelsior* L.), on soil biota and carbon dynamics differentiating between root- and leaf litter-mediated effects. The influence of beech and ash seedlings on carbon and nitrogen flow was investigated using leaf litter enriched in ^{13}C and ^{15}N in double-split-root rhizotrons planted with beech and ash seedlings as well as a mixture of both tree species and a control without plants. Stable isotope and compound-specific fatty acid analysis (^{13}C-PLFA) were used to follow the incorporation of stable isotopes into microorganisms, soil animals and plants. Further, the bacterial community composition was analyzed using pyrosequencing of 16S rRNA gene amplicons. Although beech root biomass was significantly lower than that of ash only beech significantly decreased soil carbon and nitrogen concentrations after 475 days of incubation. In addition, beech significantly decreased microbial carbon use efficiency as indicated by higher specific respiration. Low soil pH probably increased specific respiration of bacteria and suggests that rhizodeposits of beech roots induced increased microbial respiration and therefore carbon loss from soil. Compared to beech $\delta^{13}C$ and $\delta^{15}N$ signatures of gamasid mites in ash rhizotrons were significantly higher indicating higher amounts of litter-derived carbon and nitrogen to reach higher trophic levels. Similar $\delta^{13}C$ signatures of bacteria and fine roots indicate that mainly bacteria incorporated root-derived carbon in beech rhizotrons. The results suggest that beech and ash differentially impact soil processes with beech more strongly affecting the belowground system via root exudates and associated changes in rhizosphere microorganisms and carbon dynamics than ash.

Key words*: ^{13}C, ^{15}N, bacteria, carbon cycling, decomposition, fungi, nitrogen, soil food web*

6.2 INTRODUCTION

Soils store twice as much carbon as plants and the atmosphere together thereby forming an important component of the global carbon cycle (Schlesinger and Andrews, 2000). However, the way carbon is processed and how carbon dynamics are controlled still is not well understood. Knowledge on factors changing the flux of carbon from plants into the soil and controlling its turnover is of significant importance especially in face to global warming (McKinley et al., 2011).

In terrestrial ecosystems, 90% of the annual biomass produced by plants enters the dead organic matter pool forming the basis of the decomposer system in soil (Gessner et al., 2010). Plant carbon enters the soil via two pathways: dead organic matter (leaf litter and dead roots) and root exudates. Soil chemical properties are mainly influenced by parent material and mineralogy but also by leaf litter forming the major resource of soil biota responsible for decomposition processes (Reich et al., 2005; Jacob et al., 2009; Langenbruch et al., 2012). Litter quality strongly influences soil pH, as calcium and magnesium of the litter compete with H+ and Al_3^+ for exchange sites on soil particle surfaces or organic matter (Reich et al., 2005). As a consequence, high pH often promotes higher microbial biomass resulting in higher soil respiration, mineralization and decomposition (Swift et al., 1979; Wardle, 1998). Low mineralization and decomposition rates are associated with high C-to-N ratios and high lignin contents as it is typical for recalcitrant litter. In contrast, Pollierer et al. (2007) highlighted that in temperate forests carbon does not enter the soil food web predominantly via litter but rather via roots. Rhizodeposits comprise labile exudates (e.g., sugars, amino acids and organic acids), but also complex molecules (e.g., polysaccharides, mucilage and proteins). Labile exudates control both community structure and activity of rhizosphere microorganisms (Paterson et al., 2009). Summarizing results of 95 plant 14C labeling studies, Jones et al., (2004) estimated the loss of carbon by exudation to be equivalent to 5 - 10% of the net carbon fixed by plants and 25% of the carbon plants allocate to root growth. This supply of energy increases microbial biomass (Butler et al., 2004), acts as soil organic matter (SOM) priming agent (Bird et al., 2011) and alters the physical and chemical soil environment (Gregory, 2006). Microbial communities in rhizosphere and bulk soil are therefore responsible for root exudate-mediated changes in soil

processes (Söderberg et al., 2004; Paterson et al., 2007). Since plant species differ in the quality and quantity of exudates (Jones et al., 2004), soil carbon dynamics are likely affected by plant species identity and diversity (Grayston et al., 1998; Steinbeiss et al., 2008).

Decomposition studies report both individual effects of individual plant species (Jacob et al., 2009) and positive mixing effects (Gartner and Cardon, 2004; Hättenschwiler et al., 2005). Until today, however, studies investigating the influence of plant diversity on belowground dynamics in forests are scare (but see Meinen et al., 2009) and most often only consider the effect of aboveground plant residues (Hättenschwiler and Gasser, 2005; Jacob et al., 2009, 2010). To what extent belowground processes mediated by roots and root exudates affect soil organisms and thereby carbon dynamics remains largely unknown. This lack of knowledge is unfortunate as 60% of the terrestrial carbon is bound in forests and its contribution to global carbon cycling is of fundamental importance (McKinley et al., 2011).

To improve knowledge on carbon dynamics in forest soils from a root perspective we used the common temperate broad-leaved tree species European beech (*Fagus sylvatica* L.) and European ash (*Fraxinus excelsior* L.) to differentiate between general and species-specific effects of living roots on soil organisms and decomposition of litter material in soil. Beech is the dominant tree species in many Central European deciduous forests. Ash often is associated with beech forests and is expected to increase in dominance in a warmer and drier climate (Broadmeadow and Ray, 2005). Life history traits of beech and ash differ strongly, e.g. speed of growth, root morphology, litter quality, mycorrhizal association, and nutrient, water and light use efficiency (Grime et al., 1997; Emborg, 1998). Beech has higher specific root tip abundance, specific fine root surface area (SRA) and specific fine root length (SRL), whereas ash roots are characterized by higher mean fine root diameter (Meinen et al., 2009). Roots of beech are colonized by ectomycorrhizal (EM) fungi and those of ash by arbuscular mycorrhizal (AM) fungi which differ in nutrient acquisition strategies (Smith and Read, 2008). Beech tolerates soil pH from acid to highly alkaline, while ash is restricted to soils of high base saturation (Weber-Blaschke et al., 2002). Litter of beech at more acidic sites has high C-to-N ratio >50 and high lignin content, while

ash litter is regarded as high quality litter due to its low C-to-N ratio of about 28 and low lignin content (Jacob et al., 2010).

For allowing access to the root system and to investigate interactions between both tree species, beech and ash seedlings were planted into double split-root systems. The systems allowed dissecting root associated processes and belowground interactions between beech and ash. Carbon and nitrogen fluxes in soil were traced following the incorporation of ^{13}C and ^{15}N from labelled ash litter into soil, bacteria, fungi, soil animals and plants. Ash litter was used to follow the uptake of resources from high quality litter materials by beech and ash as compared to more recalcitrant soil resources.

We hypothesized that (1) beech and ash differentially affect the structure of the microbial community thereby modifying soil processes and plant nutrient capture. Differences in microbial community structure are expected to (2) result in differential decomposition of labeled ash litter and differential mobilization of nutrients from the litter. Further, we expected (3) modifications of the soil microorganisms community and soil processes to be most pronounced in the mixed treatment with both tree species present due to a complementary effect on soil microorganisms and soil processes.

6.3 MATERIALS AND METHODS

6.3.1 Rhizotrons

Double split-root rhizotrons were used to separate root systems of two tree seedlings into compartments with root strands of one individual seedling at each side and a shared root compartment in the centre where root strands of both tree seedlings could interact (Fig. 6.1). We focused on the middle compartment where the two root strands grew together. The central compartment had a volume of 7.6 L and side compartments half the volume of 3.8 L. Rhizotrons were 90 cm high and 64 cm wide, and were built from anodized aluminum covered at the front with a 10-mm Perspex plate. They were tilted at 35° to direct roots growing along the Perspex plate. The Perspex plate was covered with black scrim to ensure that roots grow in darkness. Rhizotrons were divided into six soil depth sections (I-VI). Every soil depth contained four experimental sites (ES), two in the centre and two at the sides

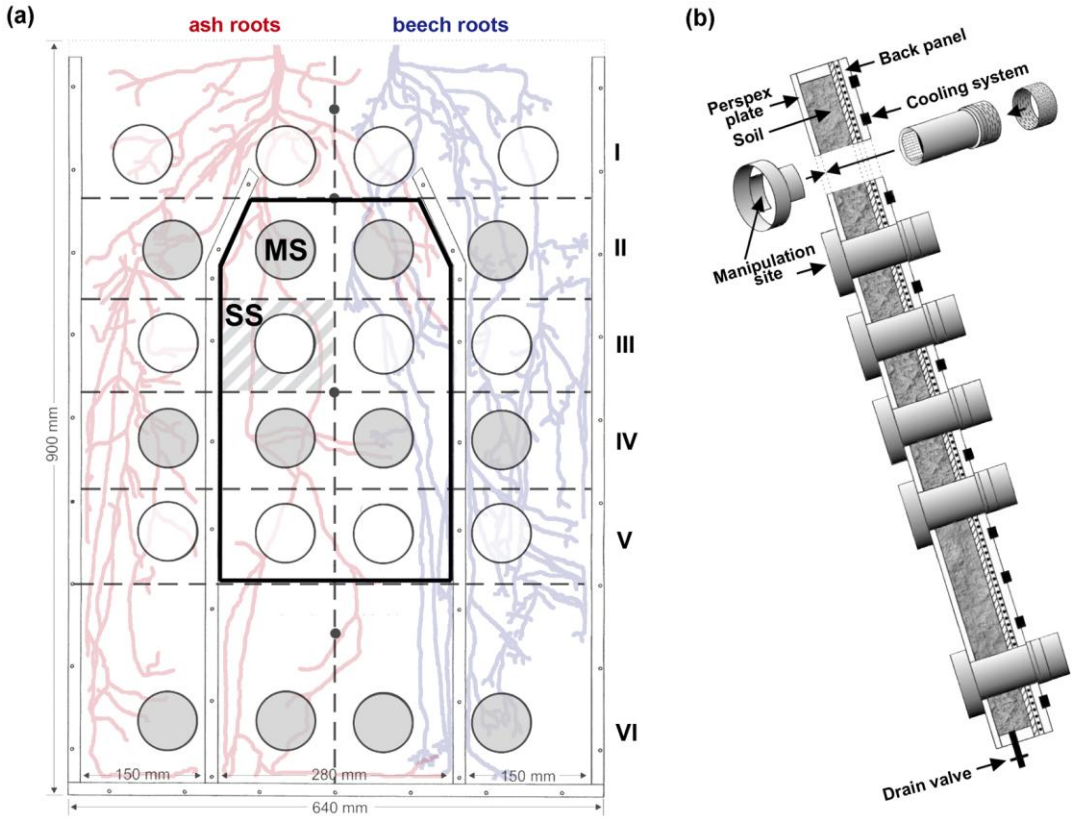

Fig. 6.1. Scheme of double split-root rhizotrons. (a) Front view of mixed species treatments with ash (left) and beech (right) roots interacting in the central compartment. Circles represent experimental sites (ES) with soil (open circles) or soil-litter mixture (grey circles). The shaded area refers to the surrounding sampling site (SS). Roman numerals indicate soil depths (I-VI). The bold rim in the central compartment from soil depth II to VI represents the sampling area. Black dots along the central dashed line refer to the position of temperature sensors. Dashed lines mark the sampling grid. (b) Side view of the double split-root rhizotron and assembly of ES. Tubes inside ES can be withdrawn to fill them with litter and/or soil. A water flux based cooling system is installed at the back panel. A valve allowed drainage of the rhizotrons.

(Fig. 6.1). The back side of the rhizotrons was equipped with a cooling system keeping the temperature at a constant level of 20 ºC over the whole soil column. Climate conditions were set to 20 °C air temperature, 70% relative air humidity and 10 h daylight in winter and 14 h in summer. The tree seedlings were illuminated (EYE Lighting, Clean Ace, Mentor, OH, USA) ensuring a minimum PPFD of 200 ± 10 µmol m^{-2} s^{-1} from June 2009 to October 2010. The experiment lasted for 475 days, i.e., plants were harvested after the second season.

Soil and plants

The soil was taken from a mixed temperate broadleaf forest dominated by F. sylvatica, *F. excelsior* and *Tilia cordata* in Central Germany (Hainich forest, 51°04' N 10°30' E, about 350 m a.s.l) from a depth of 0 - 10 cm after removing the litter. The soil type was a Stagnic Luvisol (IUSS Working Group WRB 2007; 1.8% sand,

80.2% silt and 18.1% clay) and free of carbonate (<0.02% of total carbon) with a pH(H_2O) of 4.56 ± 0.03 and a gravimetric water content at date of sampling of 22.7%. Initial total carbon amounted to 19.2 ± 0.3 g kg^{-1} dry weight, initial total nitrogen averaged 1.56 ± 0.01 g kg^{-1} dry weight and base saturation was 22.9 ± 1.3%. Each rhizotron was filled with 15.2 L of sieved soil (1 cm mesh) containing natural microflora and soil fauna. Volumetric soil water content was monitored three times a week with a TDR measurement device (Trime-FM, IMKO, Ettlingen, Germany), and kept at constant level by adding distilled water. Soil temperature was measured with NTC thermistors (Epcos, Munich, Germany), arranged vertically in the centre of the rhizotrons, at soil depths of 8, 20, 42.5 and 70.5 cm and in 2 cm distance of the Perspex plate. Data were recorded in 15-min intervals with a CR1000 data logger (combined with two AM416 Relay Multiplexer, Campbell Scientific Inc., Utah, USA).

In spring 2009, beech (*F. sylvatica*) and ash (*F. excelsior*) seedlings with comparable root biomass were excavated in the Hainich forest with intact soil cores to preserve the root system. Initial shoot height was 23.1 ± 1.2 and 17.9 ± 1.1 cm, and root length was 12.1 ± 0.7 and 15.4 ± 1.2 cm for beech and ash seedlings, respectively. At the start of the experiment, ash had significantly higher fine root biomass than beech, but tree species did not differ significantly in total root and total aboveground biomass (Table 6.1). Before planting, the soil material adhering to the root systems was removed by watering. The remaining soil-water mixture was used to equilibrate microbial communities in soil.

Fifty-three days after planting, 1.5 g labelled ash litter was added to ES of each of the

Table 6.1. Means ± 1 SE and T- and *P*-values of plant biomass of beech and ash saplings at the start of the experiment (in g $plant^{-1}$; n = 5).

	Initial Biomass			
Biomass	**Beech**	**Ash**	**T**	***P***
Total	2.04 ± 0.46	2.13 ± 0.15	0.15	0.7122
Total aboveground	1.26 ± 0.27	1.25 ± 0.01	0.01	0.9294
Total belowground	0.78 ± 0.20	0.88 ± 0.81	0.81	0.3933
Shoots	0.88 ± 0.22	0.74 ± 0.27	0.27	0.6190
Leaves	0.38 ± 0.05	0.52 ± 2.49	2.49	0.1530
Fine roots	0.16 ± 0.05	0.41 ± **6.49**	**6.49**	**0.0343**
Coarse roots	0.64 ± 0.17	0.56 ± 0.08	0.08	0.7866

treatments, i.e., the control, beech, ash and mixed rhizotros at every second soil depth (II, IV, VI; see Fig. 6.1). Therefore, tubes were withdrawn and the empty space filled with soil or soil-litter mixture. Prior to adding ash leaves (air dried, crushed to pieces < 1 cm) were mixed with 40 g soil (air dried). The litter was labelled with ^{13}C and ^{15}N by incubating ash trees in the green house for one vegetation period with the CO_2 concentration in air elevated by adding $^{13}CO_2$ (maximum concentration 1,200 ppm) and by watering the soil with nutrient solution containing $^{15}NO_3{}^{15}NH_4$ (both 99 atom %; Euriso-top, Saint-Aubin, Essonne, France). The solution contained 0.6 mM $CaCl_2$, 0.4 m*M* $MgSO_4$, 0.01 m*M* $FeCl_3$, 0.4 m*M* K_3PO_4, 1.8 µ*M* $MnSO_4$, 0.064 µ*M* CuCl, 0.15 µ*M* $ZnCl_2$, 0.1 µ*M* MoO_3, 5 m*M* NO_3NH_4 and 0.01 m*M* H_3BO_3. The stable isotope signature of the ash litter was 146.8 ± 0.3‰ for $\delta^{13}C$ and 13,139 ± 60‰ for $\delta^{15}N$ (Table 6.2).

6.3.2 Experimental design

The experiment was set up in a two-way factorial design with the factors beech and ash (absence: "-" and presence: "+"), resulting in the following treatments with four replicates each: (a) two beech seedlings (BB), (b) two ash seedlings (AA), (c) a mixture with one beech and one ash seedling (BA or AB, depending on target tree species), and (d) an unplanted control (Co), resulting in rhizotrons without (B-: Co and AA) and with beech (B+: BB and BA), as well as rhizotrons without (A-: Co and BB) and with ash (A+: AA and AB).

6.3.3 Sampling

After After 475 days rhizotrons were harvested. They were opened in horizontal position and a sampling grid was used to identify locations for sampling, i.e., at ES and the surrounding of these sites (SS; see Fig. 6.1). Samples from the depth layers II, III, IV and V of the central compartment were analyzed. Further, as we were not interested in effects of soil depth we pooled the data from the four layers. In addition

Table 6.2. Isotopic signatures of the used soil, labeled ash litter and of the soil-litter-mixture in manipulation sites at the start of the experiment and at the end after 422 d of litter incubation (means ± 1 SE).

	Start			End	
	Soil	Litter	Soil-litter mixture	Soil-litter mixture	Difference* [%]
$\delta^{13}C$ [‰]	-26.20 ± 0.10	146.80 ± 0.32	69.00 ± 0.60	-17.44 ± 1.86	88.25
$\delta^{15}N$ [‰]	1.60 ± 0.16	13139.30 ± 59.10	6153.80 ± 0.40	577.38 ± 124.88	81.23

to soil samples, plant shoots and roots from each of the soil layers were taken for measuring plant biomass. Details on root biomass distribution along the soil depth gradient as well as on gas emissions are presented elsewhere (Fender et al., 2013).

Plants

At harvest shoot length and root collar diameter of seedlings was measured. Roots were separated from soil, washed and cleaned from adhering soil particles. To obtain overall plant biomass fine root biomass estimated from ES for mycorrhizal analysis were combined with plant biomass data from SS. Whenever possible three intact root strands of ca. 7 cm length from each tree species per compartment and soil depths were taken and digitalised on a flat-bed scanner for image analysis carried out using WinRhizo 2005c software (Régent Instruments Inc., Québec, QC, Canada) to determine specific fine root area (SRA; $cm^2\ g^{-1}$ dry matter), specific fine root length (SRL; $cm\ g^{-1}$ dry matter) and total fine root surface. Thereafter, samples were oven-dried (70°C, 48 h), weighed and milled for measurement of organic carbon (C_{org}), total nitrogen (N_{total}) as well as $\delta^{13}C$ and $\delta^{15}N$ signatures (Delta C, Finnigan MAT, Bremen, Germany).

Mycorrhiza

Colonization of roots at ES by mycorrhiza-forming fungi was determined. Fine roots were stored in Falcon tubes with moist tissue paper at 4°C until analysis. Fine roots of beech were analyzed with a stereomicroscope (Leica M205 FA, Leica Microsystems, Wetzlar, Germany). The percentage of EM fungi colonization was calculated using the following equation:

$$\text{EM fungi colonisation [\%]} = \left(\frac{n\ mycorrhizal\ root\ tips}{n\ vital\ root\ tips} \right) \times 100$$

Fine Fine roots of ash were stored in 70% EtOH at room temperature. For determining the colonization by AM fungi roots were stained with lactophenole-blue (Schmitz et al., 1991) and stored at room temperature in 50% glycerol until microscopic inspection at 200x magnification. AM fungi colonization was calculated with the magnified intersection method of McGonigle et al. (1990) using a 10x10 grid. The abundance of vesicles, arbuscles and hyphae was calculated as percentage of mycorrhizal structures of the total number of intersections. The percentage of

vesicles was taken as relative colonization rate of AM fungi and used for further calculations.

Soil properties

Soil pH was measured in a suspension of 4 g soil and 10 ml H_2O with a Vario pH meter (WTW GmbH, Weilheim, Germany). Soil water content was measured gravimetrically after drying at 105°C for 24 h. Nitrate and ammonium concentrations were measured by extracting soil samples in 0.5 M K_2SO_4 solution (1:3 wet soil mass-to-solution ratio). Samples were shaken for 1 h and filtered through Sartorius folded filters (Sartorius Stedim, Aubagne, France). Nitrate and ammonium concentrations of filtered extracts were analyzed using continuous flow injection colorimetry (SAN^+ Continuous Flow Analyzer, Skalar Instruments, Breda, The Netherlands). Nitrate was determined by copper cadmium reduction method (ISO method 13395) and ammonium was quantified by Berthelot reaction method (ISO method 11732). C_{org}, N_{total} as well as $\delta^{13}C$ and $\delta^{15}N$ values were measured after grinding soil samples with a disc mill. Samples were analyzed with a coupled system consisting of an elemental analyzer (NA 1500, Carlo Erba, Mailand) and a mass spectrometer (Delta C, Finnigan MAT, Bremen, Germany).

Microbial respiration

Basal respiration (BAS), microbial biomass (C_{mic}), and specific respiration (qO_2) were measured by substrate-induced respiration (SIR), i.e., the respiratory response of microorganisms to glucose (Anderson and Domsch, 1978). Before measurement, roots were removed and soil samples were sieved (2 mm). Measurements were done using an automated O_2 microcompensation system (Scheu, 1992). BAS of microorganisms reflected their averaged oxygen consumption rate without the addition of glucose within 10-30 h after attachment of the samples to the analysis system. Subsequently, 4 mg glucose g^{-1} soil dry weight was added as aqueous solution to the soil samples. The mean of the three lowest hourly measurements within the first 10 h was taken as the maximum initial respiratory response (MIRR). C_{mic} (µg C g^{-1}) was calculated as 38 x MIRR (µl O_2 g^{-1} soil dry weight h^{-1}) according to Beck et al., (1997). Microbial specific respiration qO_2 (µl O_2 mg^{-1} C_{mic} h^{-1}) was calculated as BAS/C_{mic}.

Fatty acid analysis

Before extraction of lipids, soil samples were sieved (2 mm) and root and litter pieces were removed. Lipid extraction followed Frostegård et al. (1991). Bacterial biomass was estimated using the following PLFAs: a15:0, i15:0, i16:0, 16:1ω7, i17:0, cy17:0 and cy19:0; the PLFA 18:2ω6,9 was used as fungal biomarker (Ruess and Chamberlain, 2010). A gas-chromatography-combustion-isotope-ratio-monitoring-mass spectrometer (GC-C-IRM-MS) using Thermo Finnigan Trace GC coupled via a GP interface to a Delta Plus mass spectrometer (Finnigan, Bremen, Germany) was used to determine the isotopic composition of individual PLFAs. Fatty acid identification was verified by GC-MS using a Varian CP-3800 chromatograph coupled to a 1200L mass spectrometer and a fused silica column (Phenomenex Zebron ZB-5MS, 30 m, 0.25 µm film thickness, ID 0.32 mm) and helium as carrier gas.

Pyrosequencing

DNA 16S rRNA as well as the 16S rDNA were co-isolated to capture the active and the present microbial community; 2 g soil were extracted from control, beech and ash treatments using the RNA PowerSoil™ Total RNA Isolation Kit and DNA Elution Accessory Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA). Residual DNA contaminations in RNA extracts were removed using the TURBO DNA-free™ Kit (Ambion Applied Biosystems, Darmstadt, Germany). RNA was concentrated using the RNeasy MiniElute Kit (QIAGEN, Hilden, Germany). The nucleic acid concentration was estimated using a NanoDrop ND-1000 spectrophotometer (Peqlab Biotechnologie GmbH, Erlangen, Germany).

The V2-V3 region of the 16S rRNA was reverse transcribed using the SuperScript™ III reverse transcriptase (Invitrogen, Karlsruhe, Germany). As template 100 ng of the DNA-free RNA were applied. The resulting cDNA as well as the extracted DNA was amplified in triplicate using the Phusion® Hot Start High-Fidelity DNA polymerase (FINNZYMES, Espoo, Finland) as described by Nacke et al., (2011).

The following barcoded primer set was used for reverse transcription and amplification, containing the Roche 454 pyrosequencing adaptors (underlined): V2for 5'-

CTATGCGCCTTGCCAGCCCGCTCAGAGTGGCGGACGGGTGAGTAA-3' and V3rev 5'-CGTATCGCCTCCCTCGCGCCATCAGCGTATTACCGCGGCTGCTG-3' modified from Schmalenberger et al., (2001).

The PCR products were treated and purified as described by Nacke et al., (2011). All kits were used as described in the manufacturer´s instructions. The Göttingen Genomics Laboratory determined the sequences of the partial 16S rRNA genes using a Roche GS-FLX 454 pyrosequencer (Roche, Mannheim, Germany) according to the manufacturer´s instructions for amplicon sequencing.

Sequences shorter than 300 bp were removed from the dataset. To minimize the bias introduced by pyrosequencing due to decreasing read precision at the end of the reads denoising was carried out using Denoiser 0.91 (Reeder and Knight, 2010). OTU determination was performed using uclust OTU picker 1.2.22q (Edgar, 2010) at genetic divergence of 3%, 5% and 20% according to Schloss and Handelsman (2005). The resulting datasets have been deposited in the GenBank short-read archive under accession number SRA050002.

Soil animals

Soil not needed for other analysis was taken to extract soil animals by heat (Kempson et al., 1963). Animals were conserved in saturated NaCl solution and kept at -10°C until analysis. The gamasid mite *Hypoaspis aculeifer* (G. Canestrini, 1884) was taken for stable isotope analysis as it occurred in sufficient numbers for the analysis. Twenty adult mites were weighed into tin capsules and dried at 40°C for 24 h. Samples were analyzed as described above.

6.3.4 Statistical analysis

Two-wayTwo-way ANOVA was used to test for main effects of beech (B- and B+), ash (A- and A+) and their interactions with data of the four soil depths pooled. To detect differences in plant biomass and mycorrhizal colonization contrasts were calculated in a GLM using pairwise *t*-test to account for dependence in mixed rhizotrons. U-Test was used for analyzing the number of root tips. Treatments in beech-only rhizotrons (BB) were compared to ash-only (AA) and beech-ash mixture (BA). Ash (AA) was also compared with beech-ash mixture (AB). Statistical analyzes were done using SAS 9.2 (SAS Institute; Cary, NC, USA).

Discriminant function analysis (DFA) was used to analyze pyrosequencing data as well as fatty acid patterns combined with microbial respiration and soil chemical data. Differences of the bacterial composition in beech and ash rhizotrons and the control were calculated using multi-dimensional scaling (MDS) to reduce dimensions in the dataset. DFA and MDS were calculated using STATISTICA 7.0 for Windows (StatSoft, Tulsa, USA, 2001).

Means were compared using Tukey's Honestly Significant Difference test ($P < 0.05$). Data on plant biomass, isotopic signatures, SRA, SLR, number of fine root tips, NO_3^-, NH_4^+, C_{org}, N_{total}, microbial respiration and PLFA content were log-transformed and percentage data, i.e., colonization rate of mycorrhiza, were arcsine-square root transformed prior to statistical analyses to improve homogeneity of variance. Means given in text and tables are based on non-transformed data.

6.4 RESULTS

6.4.1 Plants and mycorrhizae

After After 475 days, total biomass of tree seedlings in BB rhizotrons was significantly lower than in AA and BA rhizotrons (Table 6.3). Fine and coarse root biomass were significantly lower in BB rhizotrons compared to that of seedlings in AA (-69%) and BA rhizotrons (-62%) resulting in significantly lower total root biomass. Total biomass, total root biomass and coarse root biomass of seedlings in mixtures exceeded that of seedlings in monocultures, but this increase was only significant for beech (60%, 62%, 70%, respectively); biomass of ash seedlings in mixture increased by 11%, 17% and 23%, respectively.

$\delta^{13}C$ and $\delta^{15}N$ signatures in fine roots were significantly lower in BB than those in AA rhizotrons (Table 6.3; see Table S.6.1 for atom% values). SRA and SRL did not differ significantly between tree species but tended to be higher in beech (BB vs AA: + 6% and +68%, respectively), especially in the mixture (BA vs AB: +24% and 79%, respectively). Generally, fine root tips of tree seedlings increased in mixed rhizotrons, especially beech in mixed rhizotrons had a significantly higher number of root tips than beech in monoculture by +89% compared to ash in mixed rhizotrons and ash in monoculture by +54%. Mycorrhizal colonization of roots of beech in BB rhizotrons was significantly lower than that of roots of ash in AA rhizotrons,

however, as beech and ash are colonized by different types of mycorrhiza the differences have to be interpreted with caution. Beech did not influence the colonization rate of ash by arbuscular mycorrhiza (AA vs AB; + 2 %), whereas ash increased the colonization of beech by ectomycorrhiza (BB vs BA; + 45 %) although the effect was not significant (Table 6.3).

6.4.2 Soil properties

In general, the studied soil properties were strongly affected by beech and not by ash with interactions between tree species also being not significant (Table 6.4). Soil pH was significantly lower in B+ (4.54 ± 0.08) than in B- rhizotrons (4.80 ± 0.06). In presence of beech, C_{org} and N_{total} were significantly decreased by -7% and -6%, respectively, but NO_3^- and NH_4^+ concentrations remained unaffected. Further, $\delta^{13}C$ and $\delta^{15}N$ of bulk soil were significantly lower in B+ (-24.46 ± 0.32‰ and 127.04 ± 19.95‰, respectively) compared to B- rhizotrons (-22.24 ± 0.78‰ and 265.25 ± 48.79‰, respectively). Generally, after 422 days of litter incubation, the signatures of $\delta^{13}C$ and $\delta^{15}N$ within the soil-litter-mixtures decreased strongly by Δ 86 $\delta^{13}C$ and Δ 5576 $\delta^{15}N$ respectively (Table 6.2).

Table 6.3. Means ± 1 SE for plant parameters per plant in rhizotrons planted with beech trees (BB), ash trees (AA), beech trees in mixture (BA) and ash trees in mixture (AB) as well as GLM table of contrasts after 475 days.

	BB (pure beech)		**AA** (pure ash)		**BA** (beech in mixture)		**AB** (ash in mixture)		**Pure beech (BB) vs. pure ash (AA)**		**Pure beech (BB) vs. beech in mixture (BA)**		**Pure ash (AA) vs. ash in mixture (AB)**	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	***F***	***P***	***F***	***P***	***F***	***P***
Biomass [g dry weight] per plant														
Total	4.52	± 0.79	12.08	± 1.69	11.33	± 2.03	13.50	± 2.33	**8.82**	**0.0117**	**6.39**	**0.0266**	0.00	0.9518
Total aboveground	1.85	± 0.38	3.34	± 0.86	4.29	± 1.01	3.03	± 0.43	4.20	0.0629	2.91	0.1138	1.11	0.3128
Total root	2.67	± 0.50	8.74	± 1.08	7.04	± 1.08	10.47	± 2.15	**9.52**	**0.0094**	**6.96**	**0.0217**	0.28	0.6035
Fine roots	0.78	± 0.18	2.38	± 0.30	1.80	± 0.27	2.27	± 0.44	**9.14**	**0.0106**	4.60	0.0532	0.09	0.7669
Coarse roots	1.89	± 0.35	6.36	± 0.87	5.24	± 0.82	8.21	± 1.91	**7.95**	**0.0154**	**6.50**	**0.0255**	0.59	0.4557
$\delta^{13}C$ [‰] plant fractions														
Shoot	-29.09	± 0.32	-28.07	± 0.28	-27.90	± 0.22	-27.40	± 0.26	**5.14**	**0.0426**	**7.00**	**0.0214**	2.12	0.1708
Leaves	-29.62	± 0.56	-29.26	± 0.27	-29.29	± 0.44	-29.83	± 0.20	0.30	0.5955	0.25	0.6287	0.75	0.4029
Fine roots	-27.64	± 0.34	-25.60	± 0.85	-27.49	± 0.19	-25.56	± 0.23	**8.27**	**0.0139**	0.04	0.8402	0.01	0.9395
Coarse roots	-28.35	± 0.31	-25.74	± 0.76	-27.15	± 0.31	-25.92	± 0.32	**12.86**	**0.0037**	2.78	0.1215	0.06	0.8162
$\delta^{15}N$ [‰] plant fractions														
Shoot	171.27	± 30.67	260.05	± 66.16	154.54	± 18.34	154.40	± 26.76	0.87	0.3701	0.07	0.8018	2.15	0.1682
Leaves	192.42	± 32.67	316.50	± 43.37	166.67	± 23.49	228.28	± 15.10	**5.34**	**0.0394**	0.55	0.4741	1.98	0.1853
Fine roots	209.02	± 41.75	396.07	± 99.34	148.85	± 17.63	214.48	± 22.80	**4.77**	**0.0496**	1.35	0.2674	4.07	0.0666
Coarse roots	193.66	± 27.78	390.78	± 78.87	178.50	± 12.60	257.86	± 19.23	**9.34**	**0.0100**	0.10	0.7630	2.81	0.1196
SRA [$cm^2\ g^{-1}$]														
Fine roots	485.16	± 15.36	456.49	± 42.70	509.00	± 54.07	410.65	± 64.00	0.23	0.6385	0.05	0.8271	0.78	0.3950
SRL [$cm\ g^{-1}$]														
Fine roots	2374.80	± 221.17	1414.42	± 168.82	3235.44	± 848.14	1810.83	± 450.85	2.89	0.1150	0.50	0.4947	0.20	0.6596
Fine root tips														
Total number	1623.50	± 230.01	2299.00	± 419.58	3072.50	± 207.37	3543.75	± 107.79	-0.48	0.9970	**-13.16**	**0.0000**	2.13	0.1750
Mycorrhiza [%]														
Colonization rate	37.81	± 8.58	81.82	± 5.17	54.80	± 6.51	83.54	± 2.87	**27.50**	**0.0002**	3.07	0.1053	0.04	0.8481

SRA: specific root area; SRL: specific root length.

*Note that the different type of mycorrhiza in beech and ash demanded for special counting techniques, thus direct comparisons have to be treated with caution but allow comparison with trees in the mixture.

6.4.3 Microorganisms

C_{mic} C_{mic} was not significantly affected by tree species and averaged over all treatments 141.25 ± 4.93 µg C g^{-1}. However, qO_2 was significantly higher in B+ (0.0101 ± 0.003 µl O_2 mg^{-1} C_{mic} h^{-1}) than B- rhizotrons (-16%, Table 6.4), which was due to marginally higher BAS in B+ (1.39 ± 0.08 µl O_2 h^{-1} g^{-1}) compared to B- rhizotrons (-15%).

The ratio of fungal-to-bacterial marker PLFAs was significantly higher in B+ (0.061 ± 0.007) than in B- rhizotrons (-53%) as the fungal biomass was higher in B+ (0.43 ± 0.08 nmol g^{-1}dry weight) than in B- rhizotrons (-47%), whereas bacterial biomass remained unaffected.

Bacterial and total PLFA content were not significantly affected by the treatments and averaged 6.67 ± 1.67 and 7.00 ± 0.53 nmol g^{-1}dry weight, respectively. The $\delta^{13}C$ values of the fungal marker PLFA 18:2ω6,9 were significantly lower in B+ (-29.93 ± 2.00‰) than in B- rhizotrons (-18.75 ± 3.60‰). Also, weighted $\delta^{13}C$ values of bacterial PLFAs were lower in B+ (-26.28 ± 0.97‰) than in B- rhizotrons (-24.40 ± 0.84‰), whereas in A+ rhizotrons (-24.87 ± 1.01‰) they tended to be higher than in A- rhizotrons (-25.82 ± 0.89‰). In general, ash did not significantly influence $\delta^{13}C$ values of marker PLFA (Table 6.4; see Table S.6.2 for atom% values).

DFA suggests strong similarity in the composition of PLFAs in BB and BA rhizotrons. Both treatments differed strongly from the AA and the control treatment (Fig. 6.2). Differences were due to low amounts of gram-negative (cy17:0) and gram-positive bacteria (i17:0), higher fungal biomass and low pH (Table 6.5, 6.6). Pyrosequencing of the bacterial community revealed high overlap of bacterial phyla and species with little differences among the treatments (Fig. 6.3).

6.4.4 Gamasid mites

The $\delta^{13}C$ and $\delta^{15}N$ from the added ash litter was incorporated into basal species of the soil food web as indicated by the label in the predatory mite *H. aculeifer* (Table 6.4; see Table S.6.1 for atom% values). The signatures suggest that incorporation of label was most pronounced in mixed rhizotrons (significant interaction between beech and ash) followed by AA, BB and control rhizotrons. Abundances of soil

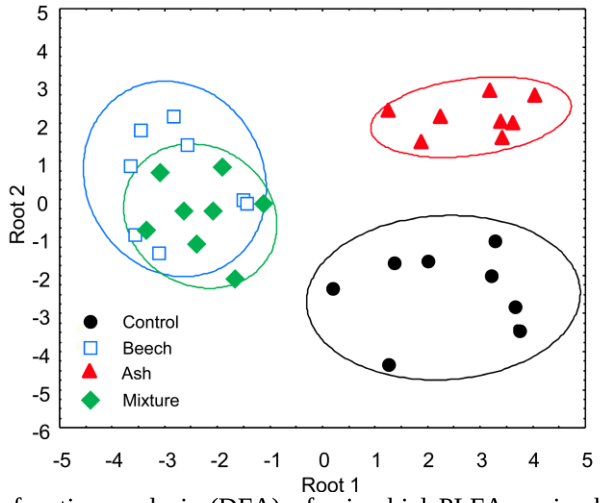

Fig. 6.2. Discriminant function analysis (DFA) of microbial PLFAs, microbial respiration and soil properties in rhizotrons without trees (control), with beech, ash and a mixture of beech and ash. Wilks' Lambda: 0.016480, F (54,33) = 1.85, *P* = 0.0296. Ellipses represent confidence intervals at *P* = 0.05.

animal taxa extracted by heat, i.e., collembolans, gamasid and oribatid mites as well as earthworms, generally did not differ among treatments (Table S.6.3).

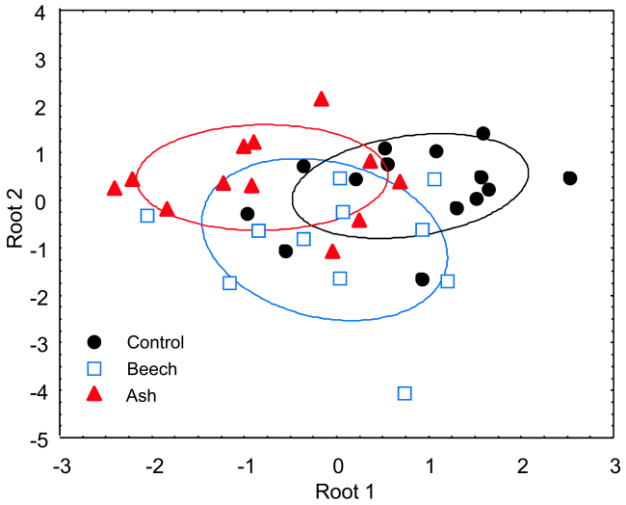

Fig. 6.3. Discriminat function analysis (DFA) of bacterial phyla based on pyrosequencing of 16S rRNA in rhizotrons without trees (control) and with beech and ash saplings after reducing data to six dimensions by multidimensional scaling (MDS). Wilks' Lambda: 0.499576; $F_{(12,60)}$ = 2.07; *P* = 0.0325. Ellipses represent confidence intervals at *P* = 0.05.

Table 6.4. Means ± 1 SE for soil and microbial parameters and on signatures in gamasid mited as influenced by beech (B) and ash (A) as well as the ANOVA table of F- and P-values in rhizotrons after 475 days (n=4). Atom% values of soil C and N, PLFA and gamasid mites are provided in Table S6.2.

	Beech absent (B-)				**Beech present (B+)**				**Beech (B)**		**Ash (A)**		**B × A**	
	Ash absent (A-) (Control)		**Ash present (A+)** (Ash)		**Ash absent (A-)** (Beech)		**Ash present (A+)** (Mixture)							
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	***F***	***P***	***F***	***P***	***F***	***P***
Soil data														
pH (H_2O)	4.78	± 0.12	4.83	± 0.05	4.53	± 0.14	4.55	± 0.11	**5.77**	**0.0334**	0.11	0.7436	0.02	0.8944
$N\text{-}NO_3^-$ [mg kg^{-1} dry weight]	41.31	± 4.96	42.88	± 3.20	39.32	± 3.83	35.14	± 5.93	1.00	0.3387	0.04	0.8532	0.62	0.4487
$N\text{-}NH_4^+$[mg kg^{-1} dry weight]	2.46	± 0.93	1.47	± 0.63	1.88	± 0.72	1.86	± 0.69	0.01	0.9422	0.41	0.5360	0.38	0.5477
C_{org} [mg kg^{-1} dry weight]	1.89	± 0.04	1.91	± 0.05	1.76	± 0.03	1.77	± 0.02	**15.02**	**0.0022**	0.08	0.7829	0.02	0.8980
$\delta^{13}C$ soil [‰]	-23.27	± 0.58	-21.21	± 1.35	-24.51	± 0.57	-24.41	± 0.40	**7.54**	**0.0177**	1.73	0.2129	1.40	0.2604
N_{total} [mg kg^{-1} dry weight]	0.18	± 0.00	0.17	± 0.00	0.17	± 0.00	0.16	± 0.00	**7.82**	**0.0162**	0.24	0.6297	0.00	0.9687
$\delta^{15}N$ soil [‰]	212.18	± 55.44	318.33	± 78.47	126.29	± 37.64	127.79	± 20.99	**7.42**	**0.0185**	0.83	0.3816	0.31	0.5907
C/N	10.78	± 0.24	10.94	± 0.08	10.66	± 0.16	10.82	± 0.14	0.56	0.4677	0.98	0.3406	0.00	0.9932
Microbial respiration														
BAS [µl O_2 h^{-1} g]	1.18	± 0.09	1.18	± 0.05	1.41	± 0.07	1.36	± 0.15	4.04	0.0674	0.09	0.7674	0.19	0.6701
C_{mic} [µg C g^{-1}]	150.03	± 13.65	134.32	± 5.93	139.79	± 6.62	140.86	± 13.38	0.03	0.8643	0.48	0.5019	0.40	0.5365
qO_2 [µl O_2 mg^{-1} C_{mic} h^{-1}]	0.008	± 0.001	0.009	± 0.000	0.010	± 0.001	0.010	± 0.001	**9.00**	**0.0111**	0.14	0.7178	1.59	0.2311
PLFA [nmol g^{-1}dry weight]														
Total	7.22	± 1.32	6.03	± 1.36	6.57	± 0.55	8.19	± 0.97	0.75	0.4025	0.00	0.9619	1.11	0.3130
Bacteria	6.95	± 1.20	5.85	± 1.29	6.25	± 0.52	7.66	± 0.95	0.53	0.4801	0.01	0.9377	1.05	0.3262
Fungi	0.27	± 0.16	0.18	± 0.07	0.33	± 0.05	0.53	± 0.15	3.36	0.0916	0.18	0.6757	1.20	0.2955
Fungi/Bacteria	0.032	± 0.017	0.026	± 0.010	0.050	± 0.008	0.073	± 0.019	**5.17**	**0.0422**	0.33	0.5755	0.85	0.3752
PLFA $\delta^{13}C$ [‰]														
Total	-22.80	± 2.37	-21.49	± 2.09	-27.14	± 0.60	-23.55	± 2.51	2.43	0.1454	1.40	0.2590	0.30	0.5944
Bacteria	-24.38	± 1.47	-24.43	± 1.05	-27.25	± 0.45	-25.31	± 1.89	2.01	0.1818	0.49	0.4960	0.51	0.4871
Fungi	-21.01	± 6.61	-17.06	± 4.53	-31.59	± 0.92	-28.27	± 4.01	**7.48**	**0.0181**	0.08	0.7807	0.16	0.6941
Gamasid mites														
$\delta^{13}C$ [‰]	-23.37	± 0.86	-13.89	± 0.31	-20.19	± 1.40	-8.78	± 0.43	**20.59**	**0.0008**	**159.43**	**<.0001**	**7.80**	**0.0175**
$\delta^{15}N$ [‰]	130.14	± 23.08	713.33	± 43.37	339.07	± 37.35	1121.26	± 26.97	**25.75**	**0.0004**	**148.88**	**<.0001**	**11.93**	**0.0054**

Table 6.5. Summary of input variables of the discriminant function analysis (DFA) of the composition of PLFA of the microbial community, soil properties and microbial respiration.

		Wilks' Lambda	F (3,11)	*P*-level
Gram$^+$ bacteria	i15:0	0.0175	0.2171	0.8825
	a15:0	0.0242	1.7284	0.2188
	i16:0	0.0237	1.6062	0.2441
	i17:0	**0.0430**	**5.8991**	**0.0119**
Gram$^-$ bacteria	**cy17:0**	**0.0390**	**5.0135**	**0.0198**
	cy19:0	0.0239	1.6448	0.2358
Unspecified bacteria	16:1ω7	0.0250	1.8939	0.1891
Fungi	18:2ω6:9c	0.0298	2.9597	0.0792
Microbial respiration	BAS	0.0178	0.2972	0.8267
	C_{mic}	0.0179	0.3145	0.8146
	qO_2	0.0175	0.2325	0.8719
Soil properties	pH	0.0320	3.4554	0.0549
	NO_3^-	0.0211	1.0298	0.4170
	NH_4^+	0.0188	0.5116	0.6825
	C_{org}	0.0182	0.3726	0.7745
	N_{total}	0.0261	2.1450	0.1524
	$\delta^{13}C$	0.0221	1.2510	0.3384
	$\delta^{15}N$	0.0173	0.1733	0.9122

6.5 DISCUSSION

6.5.1 Changes in the microbial community due to rhizodeposition

Lower pH in the rhizosphere of beech likely contributed to favoring soil fungi supporting our hypothesis (1) that beech and ash differentially affect the structure of the microbial community. Acidification of the soil by beech is well known (Holzwarth et al., 2011; Langenbruch et al., 2012), however, commonly it has been ascribed to low concentrations of calcium and magnesium and high concentrations of recalcitrant compounds such as lignin in beech leaf litter (Reich et al., 2005; Hobbie et al., 2006; Hansen et al., 2009). As we excluded leaf litter fall from seedlings to the rhizotrons soil surface and uniformly placed high quality ash litter in each of the treatments, the observed differences must have been due to the activity of beech roots. Indeed, in the vicinity of beech roots concentrations of formate and acetate were increased as compared to control rhizotrons in the same experiment, whereas in the vicinity of ash roots only the concentration of acetate increased (Fender et al., 2013). The release of organic acids increases nutrient availability and this is facilitated by low pH (Jones et al., 2004); presumably, beech employs this strategy to increase nutrient mobilization and uptake. Low pH in the soil, however,

Table 6.6. Means ± 1 SE of PLFA markers (nmol g^{-1} dry soil weight) of the microbial community in rhizotrons as influenced by beech (B) and ash (A) after 475 days.

		Beech absent (B+)		Beech present (B-)	
		Ash absent (A-)	Ash present (A+)	Ash absent (A-)	Ash present (A+)
PLFA marker		(Control)	(Ash)	(Beech)	(Mixture)
Gram$^+$ bacteria	i15:0	0.92± 0.22	0.81± 0.36	1.05± 0.21	1.59± 0.35
	a15:0	1.41± 0.29	1.04± 0.34	1.40± 0.24	1.93± 0.24
	i16:0	0.70± 0.08	0.66± 0.12	0.80± 0.06	0.87± 0.06
	i17:0	0.62± 0.05	0.74± 0.14	0.42± 0.04	0.70± 0.09
Gram$^-$ bacteria	cy17:0	0.72± 0.16	0.77± 0.13	0.63± 0.06	0.84± 0.16
	cy19:0	1.22± 0.58	1.13± 0.46	1.13± 0.22	0.74± 0.26
Unspecified bacteria	16:1ω7	1.35± 0.37	0.70± 0.35	0.81± 0.21	0.98± 0.44
Fungi	18:2ω6,9	0.27± 0.16	0.18± 0.07	0.33± 0.05	0.53± 0.15

predominantly is caused by the release of H^+ by roots rather than by dissociation of organic acids (Neumann and Römheld, 1999). Notably, acidification of the soil by beech roots occurred despite a comparatively lower root biomass in beech than ash rhizotrons. However, SRA and SRL were higher in B+ rhizotrons as compared to A+ rhizotrons. This suggests that the observed modifications were partly due to changes in root physiology rather than root biomass and number of fine root tips (Lehmann, 2003). Differences in the release rates of specific exudates of the two species presumably also contributed to the observed changes.

Bacterial community composition was little affected by tree roots as indicated by analysis of 16S rRNA. The ratio of fungal-to-bacterial biomass measured with PLFA analysis increased in B+ rhizotrons and reflected the general pattern of increasing fungal dominance at low pH accounting for differences in soil processes (Aciego Pietri and Brookes, 2008; Rousk et al., 2009). Fungal biomass was measured using 18:2ω6,9 as marker PLFA (Ruess and Chamberlain, 2010; Frostegård et al., 2011) which includes EM and saprotrophic fungi (Kaiser et al., 2010). We suggest the change in fungal biomass to refer not to AM fungi, as the PLFA 18:2ω6,9 is only found in very low densities in this type of fungi (Olsson and Johansen, 2000) and since the AM colonization rate did not change. Colonization by EM fungi in beech was relatively low (46 ± 6%, pooled data from BB and BA rhizotrons). This corresponds to low colonization rates in other greenhouse and rhizotron experiments (Dučić et al., 2009; Reich et al., 2009; Winkler et al., 2010) when compared to field data (Leuschner et al., 2004; Lang et al., 2011). Low EMF colonization rate and a stronger depletion of $\delta^{13}C$ of PLFA 18:2ω6,9 in B+ rhizotrons point to SOM

decomposition suggesting that saprotrophic rather than EM fungi increased in beech rhizotrons as fine root tips and mycorrhiza were shown to have relatively similar signatures, whereas soil is stronger depleted in $\delta^{13}C$ (Eissfeller et al. submitted to SBB, Nr. 7189). We therefore suggest saprotrophic fungi to substantially contribute to changes in the fungal PLFA marker.

Combined data on PLFAs, soil properties and microbial respiration in DFA revealed high similarity of beech and mixed rhizotrons with these differing significantly from ash and control rhizotrons. The fatty acids i17:0 and cy17:0 contributed most to this separation, with lesser contributions by pH and fungal biomass. The fatty acid i17:0 is regarded as marker for gram-positive bacteria whereas cy17:0 characterizes gram-negative bacteria, the former considered to dominate in microorganisms being present in bulk soil whereas the latter in rhizosphere soil processing labile root derived carbon (Söderberg et al., 2004; Paterson et al., 2007). The relative abundance of both was lowest in BB rhizotrons suggesting that both suffered from the presence of beech roots, presumably due to beech increasing the competitive strength of saprotrophic fungi.

6.5.2 Changes in decomposition due to different tree species

Hypothesis (2) assuming that litter decomposition is differentially affected by tree species was supported by our data. Generally, stable isotope values of the litter-soil mixture in ES decreased strongly during incubation. Ash litter is known to decompose fast; in the field it disappears entirely after two years (Jacob et al., 2009). High and constant temperatures within the climate chambers (20°C) contributed to fast decomposition of the litter in the rhizotrons (Moore-Kucera and Dick, 2008). Data on higher qO_2 (this study) and higher cumulative heterotrophic CO_2 production in beech as compared to ash rhizotrons (Fender et al., 2013) suggest an overall higher stimulation of litter decomposition in beech root affected soil, i.e., higher carbon loss due to microbial respiration. High H^+ concentrations have been shown to limit bacterial growth, while low concentrations limit fungal growth (Rousk et al., 2009). Since bacterial biomass did not decrease we suggest that bacteria were not repressed but their metabolic costs increased reflected in a higher qO_2. By lowering pH beech decreased the efficiency of bacteria to use carbon for biomass production due to increased respiratory losses leading to higher carbon loss from soil. $\delta^{13}C$ values in

fungal and bacterial PLFAs were depleted most in B+ rhizotrons suggesting that bacteria and fungi incorporated less litter carbon in presence of beech roots than of ash also indicating a faster turnover of litter carbon. Further, the more depleted $\delta^{13}C$ values in fungi compared to beech fine roots suggest that fungal carbon originated from soil organic matter in beech rhizotrons, whereas higher $\delta^{13}C$ values in bacteria rather suggest bacteria to depend on root-derived carbon as their signatures resembled that of beech fine roots (Bowling et al., 2008).

Several studies found plant species identity to have stronger effects than plant diversity (De Deyn et al., 2004; Hättenschwiler and Gasser, 2005; Ball et al., 2009), as distinct plant species act as key species (Jacob et al., 2009). The strong effect of beech in this study is mediated by roots whereas ash had no effect suggesting that rhizodeposition in ash is of minor importance. Despite this low rhizosphere changes ash incorporated more litter nitrogen than beech (Lang and Polle, 2011; Schulz et al., 2011); potentially, ash is more effective in exploiting resources from fast decomposing litter such as ash leaves or by virtue of the higher root biomass production of ash in our experiment. Notably, ash seedlings incorporated more litter ^{15}N than beech seedlings supporting the conclusion that the reduced N_{total} in B+ rhizotrons was due to increased SOM decomposition and not due to plant uptake by beech. Notable the uptake of ^{15}N declined in mixture with ash. This corresponds to field observations where the N concentrations in ash declined in mixtures with other tree species and their ectomycrrhizal diversity (Lang and Polle, 2011). A higher uptake of N by ash roots was also found in a ^{15}N tracer study in the Hainich forest where ash fine roots showed a significantly higher mass-specific uptake of labeled NH_4^+ and glycine (but not of NO_3^-) than beech roots (A. Jacob, unpubl.).

6.5.3 Channeling of litter-derived carbon into higher trophic levels

Hypothesis (3) assuming that mixing of both tree species beneficially affects the microorganisms thereby stimulating carbon turnover is supported in part by our data. Generally, mixing of tree species increased plant biomass, fine root tips, SRA, SRL and mycorrhizal colonization especially that of beech seedlings but did not affect soil chemistry and microorganisms. However, soil chemistry and microbial data are point measures and do not reflect fluxes over the whole period of the experiment. As the plants are sinks for resources made available over the whole experimental time

higher plant growth in mixed rhizotrons suggests that the gross flux of resources was greater in mixed rhizotrons.

Isotope analyses of food web components are a net measure over the long experimental period. Here, we measured $\delta^{13}C$ and $\delta^{15}N$ being incorporated within the predatory mite *H. aculeifer*. $\delta^{13}C$ and $\delta^{15}N$ values of *H. aculeifer* were significantly increased in mixed and ash rhizotrons suggesting that more litter-derived carbon and nitrogen entered basal species of the soil food web which served as prey for gamasid mites, such as nematodes and collembolans feeding on bacteria and fungi. In contrast, in control and beech rhizotrons $\delta^{13}C$ values of *H. aculeifer* resembled those in the field ($\delta^{13}C$: -23.9 ± 0.76‰; $\delta^{15}N$: +2.0 ± 2.11‰; Klarner et al., 2013) suggesting low incorporation of litter-derived carbon (and nitrogen) into the prey of *H. aculeifer*. However, the turnover of belowground C in unplanted soil, i.e., the control, was numerously shown to be lower compared to planted soil (Kuzyakov, 2010; Bird et al., 2011), i.e., soil with beech trees. Low incorporation of litter resources in BB rhizotrons may point to the fast decomposition of ash litter and to the dominance of root derived resources as basis of the soil animal food web in beech forests as suggested earlier (Pollierer et al., 2007). Nevertheless, measurements of a single species, i.e., *H. aculeifer*, do not allow to predict carbon and nitrogen cycling through the whole soil food web. However, since the soil fauna composition within the rhizotrons did not differ, we suggest tree species to significantly affect the amount and the way carbon is channeled through the soil food web.

6.5.4 Conclusions

The results suggest that the effect of living roots on litter decomposition, SOM dynamics and energy channels varies with tree species identity. Rhizodeposits have the potential to change soil pH with the potential to affect the metabolic activity of microorganisms. This propagates to higher trophic levels as tree species can impact the amount of litter-derived resource entering the soil food web and on energy channels. Effects of living roots are notoriously understudied and have to be included into studies on soil C dynamics to understand carbon and nutrient cycling as well as soil food web functioning of forests.

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6.7 SUPPLEMENTARY MATERIAL

Table S6.1 Means ± 1 SE of atom% values of soil C and N, PLFA and gamasid mites as influenced by beech (B) and ash (A) in rhizotrons after 475 days.

	BB (pure beech)		AA (pure ash)		BA (beech in mixture)		AB (ash in mixture)	
	Means	SE	Means	SE	Means	SE	Means	SE
atom% ^{13}C								
Shoot	1.07383513	0.00017420	1.07495600	0.00015455	1.07514475	0.00017318	1.07568600	0.00019963
Leave	1.07326575	0.00030884	1.07365156	0.00015001	1.07362325	0.00034224	1.07303038	0.00015562
Fine roots	1.07465313	0.00018487	1.07750778	0.00046296	1.07596411	0.00014480	1.07730790	0.00017922
Coarse roots	1.07542997	0.00016796	1.07765847	0.00041694	1.07558569	0.00023790	1.07770568	0.00024885
atom% ^{15}N								
Shoot	0.42876468	0.00559039	0.46109969	0.01205151	0.42266801	0.00472628	0.42261393	0.00689963
Leave	0.43647468	0.00595216	0.48166205	0.00789685	0.42708961	0.00605381	0.44954088	0.00388855
Fine roots	0.43692704	0.00760428	0.50868574	0.01805715	0.43140529	0.00454258	0.46031359	0.00587501
Coarse roots	0.44251012	0.00506091	0.51054955	0.01435028	0.42058708	0.00324815	0.44450474	0.00495442

Table S6.2 Means ± 1 SE of atom% values of soil C and N, PLFA and gamasid mites as influenced by beech (B) and ash (A) in rhizotrons after 475 days.

	B-				B+			
	A- (Control)		A+ (Ash)		A- (Beech)		A+ (Mixture)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Soil data								
atom% ^{13}C	1.08291691	0.00068504	1.08691934	0.00267118	1.08061441	0.00267118	1.07997306	0.00267118
atom% ^{15}N	0.4995646	0.02435464	0.57079355	0.05107642	0.44495352	0.05107642	0.43470143	0.05107642
PLFA								
Total (atom% ^{13}C)	1.08072624	0.00041531	1.08215226	0.00067208	1.07597482	0.00038549	1.07989923	0.00226927
Bacteria (atom% ^{13}C)	1.07898895	0.00120338	1.07894481	0.00082096	1.07585318	0.00033924	1.07797283	0.00043242
Fungi (atom% ^{13}C)	1.08268228	0.00023938	1.08700051	0.00061554	1.07110968	0.00036185	1.07473461	0.00054633
Gamasid mites								
atom% ^{13}C	1.08010350	0.00093649	1.09026840	0.00034029	1.08358067	0.00152939	1.09606000	0.00046706
atom% ^{15}N	0.41377425	0.00841666	0.62595061	0.01575008	0.48989230	0.01359816	0.77384461	0.00976060

Table S6.3. ANOVA table of *F*- and *P*-values as well as means ± 1 SE for soil animal taxa extracted by heat from rhizotrons influenced by beech (B) and ash (A) after 475 days.

	Beech		Ash		Beech × Ash		B-			B+				
							A- (Control)		A+ (Ash)		A- (Beech)		A+ (Mixture)	
	F	*P*	*F*	*P*	*F*	*P*	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Soil taxa														
Total	1.84	0.1994	0.02	0.8840	2.67	0.1281	294.75	130.36	93.00	20.99	131.50	26.88	141.25	25.02
Collembola†	0.20	0.6614	0.70	0.4177	0.20	0.6597	61.00	37.94	46.00	15.63	59.50	30.63	65.00	9.35
Sminthurida	0.63	0.4418	2.70	0.1263	4.41	0.0576	54.00	13.36	14.50	5.48	12.25	6.84	21.50	12.07
Gamasida	2.89	0.1148	0.00	0.9889	2.22	0.1624	167.25	116.19	21.50	4.03	49.50	17.29	43.50	9.46
Oribatida	0.15	0.7023	0.28	0.6059	0.82	0.3820	10.50	2.50	8.50	2.99	9.75	2.29	10.25	0.48
Lumbricidae	0.35	0.5626	2.40	0.1474	0.08	0.7884	2.00	0.91	2.50	1.19	0.50	0.29	1.00	0.41

†Collembola without Sminthuridae

SYNOPSIS

SYNOPSIS

The main objective of the present study was to characterise species differences among *Fraxinus excelsior* L. and *Fagus sylvatica* L. saplings with respect to their root-induced effects on CO_2, CH_4, and N_2O fluxes from a temperate forest soil (Chapter 7.1). Further, this study aimed to quantify the role of roots as a complex biotic variable for the C and N cycling in forest soil and compares this effect with the impact of adding simple C and N compounds (Chapter 7.2).

7.1 SPECIES-SPECIFIC EFFECTS OF BEECH AND ASH ON THE GREENHOUSE GAS EXCHANGE OF FOREST SOIL

In the soil columns of Chapter 4, ash saplings tended to increase the CO_2 emissions from soil more than the beech saplings, whereas in the rhizotron approach (Chapter 5) the effluxes of mono-specific beech and ash rhizotrons were similar. In Chapter 5, we differentiated the total soil respiration in root-induced soil respiration, root respiration, and soil respiration. This separation was not possible in Chapter 4 because subsequent experiments were conducted with the plants and root biomass could not be quantified. The calculations in Chapter 5 revealed that the root respiration of beech roots was of minor importance for the total CO_2 release from the soil compared to the respiration of the ash roots. This lower root respiration could be explained by the lower root biomass increment and the tenedency toward lower mass-specific root respiration rates of beech as compared to ash (22 and 25 µmol CO_2 g^{-1} dw s^{-1} vs. 27 and 28 µmol CO_2 g^{-1} dw s^{-1}, in mono and mixed rhizotrons, respectively, Chapter 5). The root-derived CO_2 efflux (root respiration and root-induced heterotrophic respiration in the rhizosphere) was, however, comparable (0.13 ± 0.01 and 0.16 ± 0.03 kg C m^{-2} 324 d^{-1} in mono-specific beech and ash rhizotrons, respectively). This was found to be linked to a negligible stimulation of the biological activity in the root surroundings by the ash roots, whereas the beech roots raised the biological activity in their direct surroundings: hence, the heterotrophic respiration of the soil organisms increased (Chapter 5). The increased heterotrophic respiration under beech saplings found in Chapter 5 matches with the higher basal respiration (BAS) detected in Chapter 6. On the one hand, the ^{13}C-PLFA analysis revealed a larger fungal biomass in beech rhizotrons compared to rhizotrons

without beech. This increase did not correspond to the observed pattern of ectomycorrhizal colonisation of beech roots, which was rather low with 46% (Chapter 6). Thus, the higher fungal biomass indicated an increase of saprotrophic fungi and a higher decomposition rate under beech, which might have caused higher C losses due to increased heterotrophic respiration. As in our comparison of soil under European beech and ash, the enzyme activity of decomposers was promoted in a North American field study under *Fagus grandifolia* Ehrh. as an ectomycorrhizal tree species, whereas *Fraxinus americana* L. with arbuscular mycorrhization had no or little effect on the proteolytic, chitinolytic, and ligninolytic enzyme activity (Brzostek & Finzi, 2011). The higher fungal biomass in our study was linked to lower pH values under beech. The pH reduction might be a result of the higher release of organic acids (Chapter 5) and H^+ (Chapter 6). The lower pH seems to have exposed the soil bacteria to stress in a way that they apparently reduced their carbon use efficiency (qO_2). In addition to the higher saprotrophic activity, this might have led to a higher CO_2 loss than in ash rhizotrons having similar bacterial acitivity.

The diverging trends in CO_2 emissions from the soil under beech and ash found in Chapter 4 and Chapter 5 (lower CO_2 effluxes under beech than under ash in Chapter 4 and similar emissions in Chapter 5) might be the result of different photosynthetic activities of the saplings within the respective experiments (Kuzyakov & Gavrichkova, 2010). In Chapter 4, the saplings were included in the headspace air volume and shaded during the measurement to exclude the photosynthesis of the leaves. During the measurements in Chapter 5, the plants received the full light (200 µmol PPFD m^{-2} s^{-1}). Photosynthesis regulates the transfer of assimilates from the leaves to the rhizosphere. Therefore, photosynthesis can affect the root-derived soil CO_2 efflux consisting of (1) the root respiration and (2) the heterotrophic respiration induced by the decomposition of root necromass and the rhizodeposits. The released rhizodeposits can be directly utilised by the mycorrhizae and microorganisms in soil or indirectly stimulate the microbial decomposition of SOM (Kuzyakov & Gavrichkova, 2010). We speculate that the downward transport of assimilates in the beech saplings of the experiment of Chapter 4 was reduced due to the shading and that the rhizodeposition of the beech saplings may have decreased. This may have led to a reduced root-derived CO_2 efflux from soil planted with beech (the absolute

values of the experiments cannot be compared due to the varying experimental conditions and durations).

For a better understanding of the rhizosphere effects of tree species with consequences for soil net CO_2 efflux, further research should consider the underlying mechanisms of the observed differences in soil respiration in the neighbourhood of beech and ash roots. The differences may be on the one hand coupled to differences in root anatomy, physiology, morphology, and especially mycorrhization, and on the other hand linked to dissimilar C assimilation rates and contrasting C transfer patterns to the soil in the two species.

The stimulation of CH_4 uptake was more pronounced in soil planted with ash than with beech. The CH_4 uptake of the soil in ash rhizotrons was increased by 173% compared to the root-free control soil, whereas for soil planted with beech saplings, only non-significantly enhanced CH_4 uptake rates (increase by 22%) compared to the control were measured (Chapter 5). Moreover, we found that the presence of ash roots led to a significant reduction of the N_2O emissions, which were up to 94% lower than in root-free soil (Chapter 4). In contrast, soil planted with beech showed just slightly reduced (Chapter 4) or even similar N_2O emissions (Chapter 5) compared to the root-free soil. However, the reduction of N_2O emissions due to the presence of ash roots was more pronounced in the soil columns of Chapter 4 than in the rhizotrons of Chapter 5. The lower reduction of N_2O emissions within the rhizotrons of Chapter 5 might be linked to the photosynthetising activity of the saplings in this experiment, which was in contrast inhibited by shading in the columns of Chapter 4. Little research has been done on the possible interrelationship between photosynthetic activity and N_2O and CH_4 fluxes and the few data are resticted mostly to non-woody plants (e.g. Chen et al., 1997; Joabsson et al., 1999; Vann & Megonigal, 2003; Hatala et al., 2012). The different N_2O emission rates measured in Chapter 4 and 5 indicate that photosynthesis might be a key regulator not only of soil respiration, but also for the fluxes of N_2O and possibly of CH_4 fluxes in forest soils, which should be considered in further studies on the species-specific effects of tree species on GHG fluxes between soil and atmosphere.

The different effects of beech and ash roots on the CH_4 and N_2O fluxes in our studies cannot be completely explained by a simple quantitative effect of higher root

biomass of ash compared to beech saplings. The suggested higher N uptake with higher biomass increments by ash might be one reason for the reduced N_2O emissions and probably increased CH_4 uptake (Chapters 4, 5, and 6). Although the N uptake effect of the ash saplings (depletion of N_{total} in the soil by 1.8%) was more than counteracted by an 8% increase in N_{total} content due to the KNO_3 addition in the N-treated soil (in Chapter 4), the N_2O emissions were 96% lower than those from root-free control soil. Thus, this study showed that the N uptake may not be the only factor in the presence of ash roots explaining the high reduction of N_2O emissions. One possible other factor is soil porosity, which may be altered in dependence of the morphology of the root systems, thereby influencing gas diffusivity that regulates soil aeration to a large extent (Neirynck et al., 2000; Augusto et al., 2002). Ash developed deep-reaching tap roots, whereas fewer beech roots reached deeper soil layers and root biomass accumulated in the upper soil (up to 20 cm soil depth, in Chapter 5). But in the soil columns (Chapter 4), the soil had a depth of 10 – 12 cm and was well aerated, and in these systems, the specific effect of ash roots was observed as well. In Chapter 4, we detected lower values of the water-filled pore space (WFPS) in soil planted with ash saplings, but the reduction of 2 – 5% did not lead to an unfavourable range for N_2O emissions (Ambus et al., 2006; Ciarlo et al., 2008). As we found a lower WFPS in the upper layer of the soil columns planted with ash saplings compared to the root-free control in Chapter 4, a higher diffusivity in the upper 1 – 2 cm with a better aeration of microsites might be another physical reason for the changed N_2O and CH_4 fluxes with the presence of ash roots.

Our results in Chapter 5 suggest that the chemical changes in soil due to the release of rhizodeposits play a key role in the greenhouse gas exchange of forest soils. Rhizodeposition is known as an important factor for microbial activity and thus for microbial respiration (Walker et al., 2003; Brimecombe et al., 2007). In our rhizotron experiment (Chapters 5 and 6), the quantity and composition of organic acids (formate, lactate, acetate, and oxalate) in the soil solution tended to differ between beech and ash. The concentration of formate and the total concentration of the measured organic acids were higher in the proximity of beech roots. This seemed to have caused higher densities of decomposing microbes, especially fungi, in the soil (Chapter 6). Nevertheless, we are far from understanding how lower exudate

concentrations of ash saplings may reduce N_2O emissions and CH_4 uptake. Thus, future investigations on the species-specific rhizosphere changes of beech and ash should consider, beside the effects on soil structure, pH, redox potential, WFPS, O_2, and CO_2, the composition of the rhizosphere solution to elucidate the consequences of beech and ash roots on the gas exchange between soil and atmosphere.

Additionally to the species-specific effects, the mixing effects of beech and ash roots on the soil system and the resulting greenhouse gas fluxes should be investigated. Although the root productivity of beech roots was higher in mixed rhizotrons than in mono-specific rhizotrons, no synergistic mixing effects on soil organisms (Chapter 6) and the resulting gas exchange were found (Chapter 5).

The GHG balance of a soil depends on how large the total sum of CO_2, CH_4, and N_2O fluxes (CO_2-eq) is (Jungkunst & Fiedler, 2007). The calculation of the greenhouse gas balance of our soil from a temperate broad-leaved forest planted with beech and ash saplings under regulated climatic conditions revealed in all experiments that the GHG balance is more favourable due to ash than beech (Table 7.1). It is clear that we carried out measurements under constant climatic conditions without diurnal and annual variation, but nevertheless, the calculations indicate that a larger admixture of the fast-growing ash in beech-dominated broad-leaved forests may improve their greenhouse gas balance.

Table 7.1. CO_2 equivalents [g CO_2-eq m^{-2} d^{-1}] for the incubated homogenised topsoil material of Hainich National Park.

		Chapter 4: Experiment A	Chapter 4: Experiment B	Chapter 5
Ambient N supply	**Control**	7.44 ± 0.30^{a}	8.31 ± 2.66^{a}	4.44 ± 1.12^{a}
	Beech	.	6.33 ± 2.07^{a}	5.90 ± 0.16^{a}
	Mixed	.	4.25 ± 0.73^{a}	6.32 ± 0.58^{a}
	Ash	4.50 ± 0.33^{b}	3.70 ± 0.59^{a}	5.42 ± 0.27^{a}
Elevated N supply	**Control**	7.42 ± 0.57^{a}	.	.
	Beech	.	.	.
	Mixed	.	.	.
	Ash	3.48 ± 0.13^{b}	.	.

The different treatments are described in the respective chapters of this thesis. Significant differences between the treatments of the respective experiments are marked with lower case letters ($P < 0.05$, the Tukey-Kramer test was used for experiment A in Chapter 4, and the Wilcoxon U-test was used for the other two experiments).

7.2 CO_2, N_2O AND CH_4 GAS EXCHANGE BETWEEN SOIL AND ATMOSPHERE AS AN INDICATOR OF BIOTIC AND ABIOTIC CHANGES IN THE SOIL C AND N DYNAMICS

In the present study, the exchange of CO_2, N_2O and CH_4 between soil and atmosphere was clearly affected by beech and ash roots in different ways. In contrast, the C (C_{org}) and N (N_{total}, NO_3^-, NH_4^+) stocks of soil under beech and ash were only slightly different. It is a fact that for the detection of the same significant relative changes; i.e. differences between treatments, for low concentrations or flux ranges (e.g. CH_4 and N_2O fluxes, unit: $\mu g\ kg^{-1}\ h^{-1}$) the absolute changes are magnitudes lower and consequently easier detectable than for parameters of greater magnitudes such as the C and N content ($mg\ kg^{-1}$) of the soil. The present study showed that the greenhouse gas fluxes as dynamic processes with small-scale turnover of low concentrations in the microgram range were rapidly reacting, sensitive indicators of small biotic and abiotic changes. Until now, this kind of response has only rarely been considered as a tool for detecting alterations provoked by roots, mycorrhizae, saprotrophic fungi, the microbial community, or soil animals. In future research on biotic changes in the soil, the trace gas exchange might be used to detect subtle changes in these agents. The sensitivity of the trace gas fluxes opens up possibilities to investigate relatively small, but nevertheless relevant, changes in the C and N cycling in soil induced by recent and future climatic and biotic changes, especially in combination with ^{15}N and ^{13}C tracer studies.

Although the N addition was conducted at higher doses than the root uptake-induced N depletion by ash in the rhizosphere, the absolute amount of reduction of the N_2O emissions due to the presence of the ash roots was two times higher than the increase due to N fertilisation (Chapter 4). Additionally, the root-induced reduction of the N_2O flux could compensate for the increase in N_2O emissions due to N fertilisation. This shows the high importance of root effects on the greenhouse gas exchange of the soil, which have been neglected until now. Thus, the investigation of biotic impacts such as root growth and soil macro- and mesofauna on greenhouse gas exchange of forest soils should be intensified in future field and laboratory research. In this context, the experimental investigation of changes caused by these biota such as releasing different C compounds is viewed as a first step toward a better understanding of future changes of the C and N dynamics. Moreover, the comparison

of these biotic effects with influential abiotic factors (e.g. the role of N, P and K and of different C compounds) in laboratory systems was found to be useful to gain insights into the relevance of biotic effects, which may help to disentangle the complex interrelationships in field systems as well.

7.3 FINAL REMARKS

The present study provided insights into the contrasting species-specific effects of beech and ash roots on soil biogeochemistry, which have to be considered in addition to the effects of leaf litter. In contrast to our expectations, no mixing effects of beech and ash roots were observed. Although just little changes of C and N compounds (e.g. C_{org} , N_{total}, NO_3^- and NH_4^+ stocks) due to root growth of beech and ash saplings were observed, clear differences in the effect on greenhouse gas exchange of these two species could be shown. The large effect of ash saplings in comparison to the effect of N addition demonstrated that the role of tree roots might have been underestimated until now. Thus, the present results suggest that more information on root-induced changes of C and N trace gas fluxes especially by rhizodeposition, in particular for mature trees under field conditions, is needed to understand the role of tree species for the greenhouse gas balance of forest soils.

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Eidesstattliche Erklärung

Hiermit versichere ich die vorliegende Arbeit mit dem Titel “The rhizosphere effects of *Fagus sylvatica* L. and *Fraxinus excelsior* L. saplings on greenhouse gas fluxes between soil and atmosphere” selbstständig und unter ausschließlicher Verwendung der angegebenen Literatur, Verweise und Hilfsmittel erstellt zu haben. Verwendete Quellen wurden als solche gekennzeichnet.

Göttingen, 2. Mai 2012