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Riitta Kettunen

N_2O, CH_4 and CO_2 fluxes from agricultural organic and mineral soils grown with *Phleum pratense* and mixed *Trifolium pratense*/*P. pratense* under elevated CO_2 concentration

ACADEMIC DISSERTATION

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Key words nitrous oxide, carbon dioxide, methane, elevated carbon dioxide concentration, biomass production, *Phleum pratense*, *Trifolium pratense*

The aim of this thesis was to find out whether the fluxes of greenhouse gases increase under elevated CO_2 concentration. Fluxes of nitrous oxide (N_2O), carbon dioxide (CO_2) and methane (CH_4) were studied in greenhouse conditions. 36 mesocosms of organic (peat) or mineral (sandy soil), sown with *Phleum pratense* or mixed *Trifolium pratense/P. pratense*, were randomly distributed applying two CO_2 treatments, 360 ppm (ambient) and 720 ppm (elevated). The yield was harvested and fertilised with NPK fertiliser several times during the experiments. The dry biomass of the harvested yields and the root biomass of *P. pratense* were determined.

With mineral soil, yield and total biomass production increased under elevated CO_2, even with the low N supply, but with the organic soil, more fertiliser N was needed to obtain the CO_2 response. Root production of *P. pratense* at the end of experiments increased markedly under elevated CO_2 concentration, especially with the mineral soil. The N concentration in the above-ground dry biomass of *P. pratense* decreased at elevated CO_2, giving lower N yield in the harvested yield. By contrast, the presence of legume *T. pratense* in the mixture increased the N yield under elevated CO_2 despite the decrease in N concentration of *T. pratense*.

Photosynthesis of *P. pratense* acclimated for a higher supply of atmospheric CO_2, irrespective of the N fertilisation treatment. The total respiration rate was not markedly changed under elevated CO_2. The water content of the topsoil increased under elevated CO_2, but this had no explicit effects on CO_2, CH_4 and N_2O fluxes. Moreover, CH_4 dynamics in contrast to the N_2O fluxes, was not affected by elevated CO_2 concentration.

Elevated CO_2 concentration increased N_2O fluxes from agricultural peat and sandy soil after harvest of *P. pratense,* but this required adequate N availability and simultaneous watering or a raised groundwater table. By contrast, elevated CO_2 did not increase N_2O fluxes from sandy soil after the harvest of a mixed stand of *Trifolium/Phleum.* In fact, the N_2O fluxes from this soil were diminished under elevated CO_2 unless there was a high level of groundwater table and excess N availability. It can thus be concluded that elevated CO_2 generates higher N_2O fluxes from agricultural peat and sandy soils if water content and N availability are high enough, i.e. in conditions where denitrifying bacteria can take the benefit from the extra carbon derived from plants.

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LIST OF ORIGINAL PUBLICATIONS

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This thesis is based on the following publications and unpublished results. The publications are referred to in the text by their Roman numerals, I-IV.

I Kettunen, R., Saarnio, S. Martikainen P., Silvola, J. 2005. Elevated CO_2 concentration and nitrogen fertilisation effects on N_2O and CH_4 fluxes and biomass production of *Phleum pratense* on farmed peat soil. Soil Biology & Biochemistry 37, 739-750.

II Kettunen, R., Saarnio, S., Silvola, J. 2007. N_2O fluxes and CO_2 exchange at different N doses under elevated CO_2 concentration in boreal agricultural mineral soil under *Phleum pratense*. Nutrient Cycling in Agroecosystems 78, 197-209.

III Kettunen, R., Saarnio, S. Martikainen P. J., Silvola, J. 2006. Increase of N_2O fluxes in agricultural peat and sandy soil under elevated CO_2 concentration: concomitant changes in soil moisture, groundwater table and biomass production of *Phleum pratense*. Nutrient Cycling in Agroecosystems 74, 175-189.

IV Kettunen, R., Saarnio, S. Martikainen P. J., Silvola, J. 2007. Can a mixed stand of N_2-fixing and non-fixing plants restrict N_2O emissions with increasing CO_2 concentration? Soil Biology & Biochemistry 39, 2538-2546.

This thesis is part of the FIGARE (Finnish Global Change Research Programme) programme.
I participated in the planning of studies II-IV. I was responsible for collecting the data on gas flux and biomass in studies I-IV. Gas flux measurements were carried out with help of Matti Naakka and three Master of Science students. Root biomass collection (I-III) was executed at the Mekrijärvi Research Station, as were the measurements of oxidation and production potentials of CH_4. I was responsible for analysing the data in the studies, interpreting the results and writing the manuscripts, on which the co-authors have commented. I am the corresponding author for all the publications.

1 INTRODUCTION

1.1 Greenhouse effect

The greenhouse effect is essential for maintaining life on the surface of the Earth. Most of the solar radiation that passes through the atmosphere is absorbed by the Earth's surface, water vapour and gases. This absorbed radiation energy radiates outwards and part of the radiation is absorbed by greenhouse gases in the atmosphere, which re-emit the radiation energy in all directions, including downward onto the Earth's surface. Thus greenhouse gases trap heat within the atmosphere, warming the Earth's surface. Without the greenhouse effect, the global mean temperature near the Earth's surface would be approximately - 19°C while it is now + 14°C (IPCC 2001). Due to human activity, a new phenomenon has been recognized: enhanced greenhouse effect. A higher concentration of greenhouse gases in the atmosphere traps more infrared radiation, i.e., radiative force increases (W/m^{-2}), which further warms the Earth's surface. The enhanced greenhouse effect is the key factor driving climate change.

1.2 Greenhouse gases

Greenhouse gases are of both natural and anthropogenic origin. The primary greenhouse gases in the Earth's atmosphere are water vapour (H_2O), carbon dioxide (CO_2), nitrous oxide (N_2O), methane (CH_4) and ozone (O_3). In addition, several human-made greenhouse gases have entered the atmosphere, such as halocarbons (CFC-11, CFC-12, CFC-113), hydrofluorocarbons (HFC), perfluorocarbons (PFC) and sulphur hexafluoride (SF_6) (IPCC 2001). Since the year 1750, concentrations of CO_2, CH_4 and N_2O in the atmosphere have increased from 280 to 380 ppm (parts per million), from 715 to 1783 ppb (parts per billion) and from 270 to 319 ppb in 2005, respectively. Today's atmospheric CO_2 and CH_4 concentrations have not been exceeded during the past 650 000 years and are continuously increasing (IPCC 2007). CO_2 currently contributes 62% to global warming, while the proportion of CH_4 is 20% and of N_2O 6% (WMO 2006). In addition to the global warming potential, increasing CO_2 concentration may have a significant effect on terrestrial organic carbon (C) and nitrogen (N) cycles through changes occurring in plants. Plant reactions have ramifications for soil decomposition processes, which are linked to the Earth's nutrient cycle.

1.3 CO_2, CH_4 and N_2O exchange between a soil and the atmosphere – formation and control

1.3.1 CO_2 exchange

The principal drivers of CO_2 exchange are plants, soil micro-organisms and soil animals, by fixing and releasing CO_2 in photosynthesis and decomposition, respectively. Without the decomposition of organic matter, CO_2 does not return to the atmosphere and the nutrients would be fixed in unavailable forms for plants, and hence further primary production would be impossible (Berg and Laskowski 2006). Agricultural soils can act as a sink or source of CO_2, depending on soil type, cultivated species, cultivation techniques (Kasimir-Klemedtsson et al. 1997, Maljanen et al. 2001, Smith et al. 2001, Smith et al. 2005) and regions (Paustian et al. 1997, Smith et al. 2005).

Gross photosynthesis (P_G) indicates the total amount of CO_2 fixed by primary producers. P_G is controlled by light (photosynthetic active radiation, PAR), CO_2, temperature, nutrient and water availability (Mooney and Ehleringer 1997) as well as photosynthesising biomass and species composition (Craine et al. 2001). In agricultural

practices the availability of nutrients, especially that of N, is assured by regular fertilisation.

Fixed CO_2 is released through respiration, which functionally is divided into autotrophic and heterotrophic respiration (Trumbore 2006). Soil respiration is the sum of heterotrophic and root respiration and is the main pathway through which the CO_2 fixed by plants is released from the soil back to the atmosphere. This flow is on average 75 x 10^{15} g C/yr (Schlesinger and Andrews 2000). Soil respiration consists of root-associated activity, i.e. respiration of root cells, mycorrhizae, and rhizospheric respiration and respiration in the decomposition of soil from organic matter and litter (Kuzyakov 2006). Soil respiration rate is controlled by soil temperature, moisture, pH, oxygen supply, inorganic nutrients and clay content. The nutrient status of decomposing matter, such as C/N ratio and amount of lignin, has an impact on decomposition rate (Richards 1987). About two-thirds of terrestrial organic C is located below ground, hence changes in soil respiration rate could have a major impact on the atmospheric CO_2 concentration (Schlesinger 1977).

Ecosystem net CO_2 exchange (NEE) is the difference between gross photosynthesis and total respiration (heterotrophic and autotrophic). Organic agricultural fields are a net source of CO_2 due to the high rate of respiration, i.e. decomposition of organic matter (Kasimir-Klemedtsson 1997, Maljanen et al. 2001). NEE in mineral agricultural soils in Finland is unknown, but the results from temperate grassland ecosystems indicate a possibility of C accumulation, depending on cultivation practices (reducing or eliminating tillage) (Paustian et al. 1997, Smith et al. 2001) and soil moisture, which may be the most important factor controlling C gain (Flanagan et al. 2002).

1.3.2 CH_4 formation and consumption in soil

CH_4 fluxes are controlled by two microbial processes: CH_4 production and CH_4 oxidation (Conrad 1989). CH_4 results from an anaerobic decomposition process of organic matter by methanogens, which belong to the domain of Archaea (Woese et al.1990). Methanogens can utilise only a limited number of relatively simple substrates; H_2 + CO_2, acetate, formate, methylated compounds and primary and secondary alcohols. In most environments the two major pathways of CH_4 production are acetotrophy and CO_2 reduction by H_2 (Jones 1991, Le Mer and Rogers 2001). Methanogenic activity is inhibited by oxygen but also by electron acceptors like nitrate, nitrite, Fe (III), Mn (IV) and sulphate because these cause depletion of methanogenic substrates in anaerobic environment (Conrad 1989, Boone 1991). The activity of methanogens depends on availability of organic matter, temperature and pH (Oremland 1988, Conrad 1989, Jones 1991). Most methanogens grow optimally at neutral pH and mespohilic temperatures (+ 30–40°C), although some are active at low or higher temperatures and in acidic environments (e.g. peat) (Conrad 1989, Jones 1991).

CH_4 is consumed in soils through microbial oxidation (methanotrophy) into CO_2 (Le Mer and Rogers 2001), and in addition, nitrifiers can oxidise CH_4 (Jones and Morita 1983). Methanotrophic bacteria use CH_4 as a major C and only energy source (Topp and Hanson 1991). Oxygen and CH_4 availabilities are the main factors affecting the activity of obligate aerobic methanotrophs (Le Mer and Rogers 2001, Topp and Hanson 1991). CH_4 oxidation is inhibited by NH_4^+ (Madigan et al. 2000).

An environment can act as a sink or a source of CH_4, depending on the balance between CH_4 production and oxidation. Over 50% of global CH_4 emissions are of human origin. Agriculture, especially rice cultivation and ruminant husbandry, is one

of the major anthropogenic sources of CH_4 (IPCC 2001). Highly aerobic soils, such as mineral agricultural fields, usually consume CH_4 (Conrad 1989), but farmed organic soils can act as negligible sources or sinks of CH_4 (Kasimir-Klemedtsson et al. 1997). Nitrogen fertilisation can alter soil CH_4 dynamics, since inorganic NH_4^+ can inhibit CH_4 oxidation in agricultural soils (Crill et al. 1994, Hütsch 1998).

1.3.3 N_2O production and control

N_2O enters to the atmosphere from natural sources (e.g. oceans, wet soils, forest soils) and due to human activity (e.g. agricultural practices, biomass burning and industrial sources) (IPCC 2001). Agricultural soils are the highest anthropogenic source of N_2O, mainly due to N fertilisation and the use of N_2-fixing legumes. N_2O is produced in bacterial denitrification and nitrification processes (Firestone and Davidson 1989). Nitrification plays an essential part in the N cycle of terrestrial and aquatic ecosystems, converting ammonia (NH_3) via nitrite (NO_2^-) to the nitrate (NO_3^-), which can be denitrified (Schlesinger 1997). Nitrification depends on ammonification, a process where organic nitrogen is converted to ammonium by microbes (Stanier et al. 1979). Extra NH_4^+ is supplied to agricultural systems via fertilisation (Prosser 1989). Nitrification (Fig. 1.) is carried out mainly by chemoautotrophic bacteria.

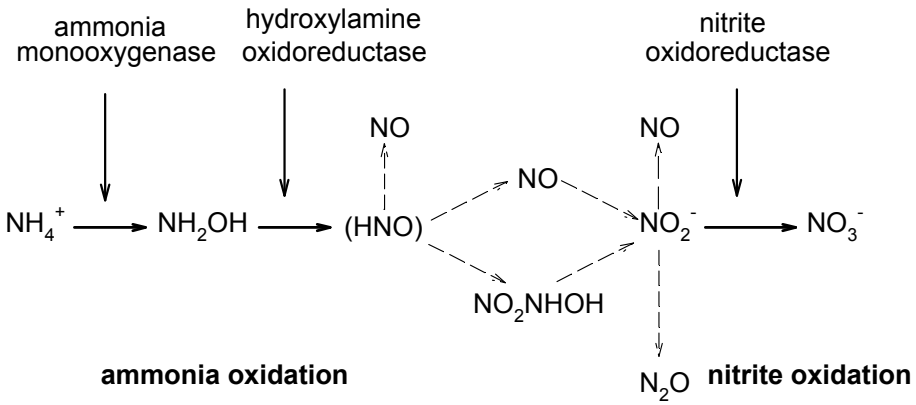

Figure. 1. A schematic presentation of the intermediates and enzymes involved in autotrophic nitrification. The dashed lines shows the possible sites for gaseous losses during the process (according to Paul and Clark 1996, Madigan et al. 2000 and Wrage et al. 2001)

Autotrophic nitrification is divided into two steps: ammonia oxidation to nitrite and nitrite oxidation to nitrate (e.g. Paul and Clark 1996, Madigan et al. 2000). Ammonia oxidisers found in soils belong to the genera *Nitrosomonas*, *Nitrosospira*, *Nitrosococcus*, *Nitrosovibrio* and *Nitrosolobus*, whereas nitrite oxidisers are found to represent two genera: *Nitrobacter* and *Nitrospira* (Paul and Clark 1996). In addition, autotrophic ammonia oxidation archae have been found in soils (Leininger et al. 2006). Several key enzymes are needed in the oxidising processes (Fig.

1). The Nitrobacteriaceae are aerobes and obligate autotrophs, which derive their C mainly from CO_2 and carbonates. The energy for the CO_2 fixation originates from NH_3 or NO_2^- oxidation (Paul and Clark 1996).

However, the autotrophic nitrifiers are not the only microbes capable of nitrifying. Heterotrophic nitrifiers including bacteria and fungi are also known. They use organic carbon as a source of C and energy (Prosser 1989). Heterotrophic nitrifiers can, in addition to NH_3, oxidise organic nitrogen, such as urea (Papen et al. 1989), hydroxylamine, amino or oxime nitrogen, aliphatic and aromatic nitro compounds and nitrite (Prosser 1989). Heterotrophic nitrification is considered to contribute to N_2O production in acidic forest soils (Killham 1990, Prosser 1989), but with present knowledge, the importance of heterotrophic nitrification in soils is still unclear. Autotrophic nitrification can produce N_2O in acidic forest soils and also in agricultural soils (De Boer and Kowlchuk 2001).

Nitrification is controlled by the availability of ammonia, oxygen and CO_2 and by pH, soil moisture and temperature. The presence of O_2 is obligatory for nitrification. Increased soil moisture limits O_2 availability, thus suppressing nitrification (e.g. Paul and Clark 1996). The optimal WFPS (Water Filled Pore Space) for nitrification in agricultural soils can be even 60–100% (Klemedtsson et al. 1988, Pihlatie et al. 2004). For nitrification, optimal soil pH is 6.6 to 8.0 (Paul and Clark 1996), but autotrophic nitrification can occur also in acid soils, pH 4-6 (De Boer and Kowalchuk 2001). Nitrification takes place under snow cover at low temperatures (Maljanen et al. 2003b), the optimum temperature being between +30–35°C (Paul and Clark 1996).

In addition to nitrification, N_2O is produced in denitrification, which is strictly coupled to nitrification. Among heterotrophic nitrifiers, bacteria *Alcaligenes* and *Thiosphera pantotropha* are able to nitrify and denitrify, depending on the availability of substrates (Castignetti and Hollocher 1984, Robertson and Kuenen 1991). Denitrification is mainly an anaerobic process, but aerobic denitrification, i.e. active denitrification in the presence of oxygen, has also been observed (Robertson and Kuenen 1991). Most denitrification is bacterial anaerobic respiration, in which nitrogenous oxides, mainly nitrate and nitrite, are reduced to dinitrogen gases, N_2O and N_2 (Tiedje 1988). Denitrification is the main process that releases dinitrogen (N_2) back into the atmosphere (Fig. 2).

Diverse bacteria are able to denitrify, i.e. reduce NO_3^- to gaseous NO, N_2O and N_2 (Fig. 2). The denitrifying micro-organisms include organotrophs, phototrophs and lithotrops (Bremner 1997). The dominant denitrifiers are organotrophs, deriving their energy from organic substrates (Tiedje 1988). These include bacteria from several genera, such as *Pseudomonas*, *Alcaligens*, *Bacillus*, *Agrobacterium*, *Flavobacterium*, *Paracoccus* (Tiedje 1988, Paul and Clark 1996). These micro-organisms are facultative anaerobes, which are able to use NO_3^- as an electron acceptor in respiration at low-oxygen or anaerobic conditions (Wrage at al. 2001). Denitrifiers are widely distributed in nature, and denitrification enzymes are persistent even in well-aerated soils (Tiedje 1988, Bremner 1997).

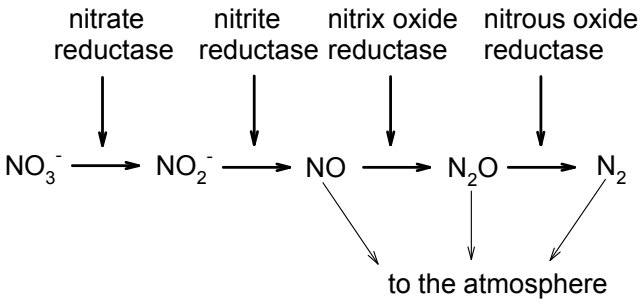

Figure 2. A schematic presentation of the intermediates and enzymes involved in denitrification (according to Madigan et al. 2000 and Wrage et al. 2001).

Denitrification is controlled in anaerobic conditions by the availability of NO_3^- and organic C, as most denitrifiers are heterotrophic bacteria. Soil pH and temperature also affect denitrification. Optimal soil pH is from 6 to 8; below pH 5, denitrification becomes slow and denitrification by organotrophs is highly restricted below pH 4. Denitrification occurs at wide range of soil temperatures, from + 5°C up to +75°C (Paul and Clark 1996). Soil NO_3^- content and pH controls the ability of soils to reduce N_2O to N_2 under anaerobic conditions. Low NO_3^- concentration retard a reduction of N_2O to N_2 by denitrifying organisms and high NO_3^- concentrations can nearly inhibit the reduction process. The inhibitory effect increases markedly with decrease in soil pH (Bremner 1997).

In addition to denitrification and nitrification, there are some others microbial processes producing N_2O, anaerobic ammonia oxidation (Wrage et al. 2001, Jetten 2001) and ammonia oxidation of methanotrophs (Topp and Hanson 1991). Chemical formation of N_2O (chemo-denitrification) is also known (Firestone and Davidson 1989, Bremner 1997). But according to present knowledge, denitrification and nitrification are the main N_2O producing processes in soils (Firestone and Davidson 1989, Wrage et al. 2001). They can occur simultaneously in soil under favourable conditions (Abbasi and Adams 2000). In fact there is a strong coupling between nitrification and denitrification in soil, as denitrification requires the nitrate/nitrite produced by nitrification (Wrage et al. 2001).

1.4 Enhanced supply of atmospheric CO_2 concentration

Emissions of greenhouse gases are strongly affected by human activity, especially by agriculture practices. Increased atmospheric CO_2 concentration affects soil processes via plant reactions. Elevated CO_2 enhances photosynthesis (e.g. Ryle et al. 1992, Ainsworth et al. 2003) and increases above and below ground biomass production (e.g. Niklaus et al. 2001, Suter et al. 2002). The water use efficiency of plants affects soil moisture (Drake et al. 1997, Niklaus et al. 1998), and C and N partitioning in plant parts can be altered (van Ginkel et al. 1997, Hill et al. 2007). Under elevated atmospheric CO_2 concentration, more new C is supplied to the soil (Hungate et al. 1997, Jastrow et al. 2005). In addition, the N_2 fixing capacity of legumes can be enhanced by elevated CO_2 (Zanetti et al. 1996), thus increasing N availability in soil.

1.5 Objectives of the study

This thesis is a part of a research consortium (AGROGAS) assessing Finnish agricultural soils as sinks/sources of greenhouse gases. AGROGAS is part of the Finnish global change research programme (FIGARE). The present study focused on process studies to find out how elevated CO_2 concentration affect N and C fluxes and plant growth in agricultural soils. Elevated CO_2 affects the water and nutrient use of the plants and C allocation to the soil, which in turn may change nitrification and denitrification activities. The more specific objectives of this thesis were to seek answers to the following questions: How the elevated CO_2 concentration

1. affect N_2O and CH_4 fluxes from agricultural organic (peat) and mineral soils (sandy loam) under *Phleum pratense* (I, III), at different N doses and groundwater table levels,
2. change soil moisture and how does this change affect the CH_4, CO_2 and N_2O fluxes from agricultural organic and mineral soils (II),
3. affect the above and below ground biomass production of *P. pratense* (I, II, III and IV) and the above ground biomass production of *Trifolium pratense* (IV),
4. affect the concentration of the total N in the above ground biomass of *P. pratense* and *T. pratense* (I, II, III and IV),
5. affect N_2O emissions from agricultural mineral soil under a mixture of *P. pratense* and *T. pratense* (IV)?

The specific hypotheses for this thesis were:

1. elevated CO_2 concentration increases N_2O fluxes from agricultural organic and mineral soils under *P. pratense* (I, II, III),
2. elevated CO_2 combined with a raised groundwater table increases CH_4 fluxes from peat soil (I) and N_2O fluxes from the peat and mineral soils (I,II,III and IV),
3. root production of *P. pratense* increases, and N concentration in above ground dry biomass decreases under elevated CO_2 concentration (I, II, III and IV),
4. the photosynthesis of *P. pratense* acclimates to an increased supply of CO_2, and the rate of total respiration (R_{TOT}) increases due to elevated CO_2 concentration (III),
5. elevated CO_2 concentration increases N_2O fluxes from sandy soil under mixed *Phleum/Trifolium* growth (IV).

2 MATERIAL AND METHODS

The effects of elevated atmospheric CO_2 concentration on C and N fluxes in agricultural soils were studied on exchange of greenhouse gases (N_2O, CH_4 and CO_2) between the soil and the atmosphere and on the above and below ground biomass production of *P. pratense* and *T. pratense*. The studies (five experiments) were carried out in controlled conditions, in four greenhouses (I, II and IV) and two growth chambers (II and III). Study II consists of two consecutive experiments.

2.1 Soils

Soils were obtained from agricultural fields in Jokioinen, southern Finland (I–IV). The characteristics of the soils differed, the organic soil (peat) having higher organic C content (23.6%) than the mineral soil (sandy

loam) (2.4%) (I–IV). The total N content was higher in the peat soil (1.1%) than in the sandy loam (0.16%) (II). Soil pH was nearly the same for both soils, 5.8 and 6.0 in the peat and the sandy loam, respectively (II). According to the FAO classification, the organic soil was Terric Histosol and the mineral soil Eutric Cambisol.

2.2 Plant species

Timothy (*Phleum pratense*) was selected because it is the most important and widely grown and cultivated forage grass in the Nordic countries (in the boreal zone). Red clover (*Trifolium pratense*) was selected as it is the most widely used legume in Finnish agriculture practice. Moreover, *P. pratense,* like *T. pratense,* have not been widely studied under elevated atmospheric CO_2 concentration, in contrast to perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) grown in the temperate regions.

2.3 Experimental arrangements

In all the experiments soil was put into 36 mesocosms that consisted of a PVC tube 10 cm in diameter and 47 cm in height. The tubes were closed with a plastic plug (I–IV). All 36 mesocosms were equally distributed, either in four greenhouses (I, II, IV) or two growth chambers (II, III) equipped with a refrigerator unit to cool bottom part of the mesocosms (Fig. 3). One half of the mesocosms (i. e. 18) was under ambient CO_2 (360 ppm) and the other half was under elevated CO_2 (720 ppm).

The air temperature in the greenhouses and the growth chamber was set at + 20°C and the temperature of the refrigerator units was set at + 15°C. The soil temperature of the mesocosms was recorded via thermosensors placed at different soil depths (I–IV). Topsoil moisture was controlled in connection with gas samplings. The level of the groundwater table was controlled on average five times during the week. The purpose was to maintain the topsoil moisture and groundwater table at the same level in all 36 mesocosms (I, II, IV) excluding the third study (III). In the third study, there were two watering treatments, i.e. in practice one half of the mesocosms were watered with an equal amount of deionised water (the same watering treatment) and the other half of the mesocosms were tended to keep equally moist with the proper amount of added water (the same moisture treatment) (III). At the end of each experiment, the water table in all the mesocosms was raised 10 – 25 cm higher than during the earlier periods, to provide suitable conditions for the denitrification process (I–IV).

The greenhouses and growth chambers were thermo-controlled, and natural light in the greenhouses was supplemented with metal halogen lamps. Air temperature, irradiation and CO_2 concentration were recorded automatically, and the flow of CO_2 from a pressure tank to the greenhouses and growth chambers was controlled in order to keep CO_2 concentrations at the adjusted level (I, III).

2.4 Fertilisation

All the mesocosms were fertilised with a commercial NPK fertiliser; N was added as NH_4NO_3 in the beginning of the experiments and after every harvest (I–IV). The amount of applied fertiliser depended on the soil type and the study arrangements (see Table 1). The mesocosms in studies I, II and IV were divided into different N fertilisation groups: low, moderate and high (excluding IV). All the mesocosms received extra N application (20 g N m^{-2}) in connection with the raised groundwater table in the last part of experiments. The moderate N treatment corresponds approximately to the fertilisation rate for grass and silage production in Finland.

2.5 Growing of *Phleum pratense* and *Trifolium pratense*

P. pratense and *T. pratense* were sown on the mesocosms in connection with fertilisation and watering, in order to ensure favourable conditions for germination and initial growth. In the experiments of I–III for all the 36 mesocosms 15 seedlings of *P. pratense* were left and in the experiment of IV, 12 seedlings of *P. pratense* and 3 seedlings of *T. pratense* were left to ensure sufficiently dense growth. During the studies the biomass was harvested (at a cutting height of 5 cm) several times and the above ground biomass was oven-dried and determined. From dry above ground biomass, the total N concentration (%) was determined by the Kjeldahl method (I–IV). After the gas exchange measurements in studies I, II and III, the soil profile (Fig. 4) at each mesocosms was divided into 5 cm slices. The roots were separated from the soil and dry weight was determined. In study IV, only the main roots of *T. pratense* were separated from the soil and the number of root nodules was observed. The thickness of the root neck was measured. After each study, the number of living branched shoots of the study plants was counted in every mesocosm (I–IV).

2.6 Gas flux measurements

2.6.1 N_2O and CH_4

Measurement of N_2O and CH_4 fluxes began after sowing and fertilisation (I–IV) using the dark, static chamber (Fig. 3) method (Crill, 1991). The gas samples were analysed within 6–16 h with a gas chromatograph (Shimazu GC-14-A, Kyoto, Japan) equipped with flame ionisation (FID) and electron capture detectors (ECD) (I–IV). Thirty-six mesocosms were measured once or twice weekly. The N_2O and CH_4 flux rates were calculated from the linear change in the gas concentrations in the chamber. The divergence of the air temperature from the set +20 °C was taken into account during the flux calculations (I–IV).

2.6.2 Measurements of CO_2 exchange

Instant CO_2 flux measurements were carried out after the measurements of N_2O efflux in studies II and III (unpublished data). The stand of *P. pratense* was maintained by clipping at a height of 18 cm. CO_2 net exchange was measured using a portable CO_2 analyser with transparent vented chambers equipped with a halogen lamp, and total agroecosystem respiration was measured with an opaque vented chamber. The rates (mg CO_2 m^{-2} h^{-1}) of net CO_2 exchange (NEE) and total agroecosystem respiration (R_{TOT}) were calculated from a linear change in CO_2 concentration during the measurement time of 150 s (II). The NEE and the R_{TOT} were also measured at the changed CO_2 concentration. In practice, one day before the measurements, the CO_2 concentration in the growth chamber was altered to be the opposite of the growing conditions of the mesocosms (II). The rate of gross photosynthesis (P_G) was estimated as a sum of CO_2 fluxes measured in light (NEE) and dark (R_{TOT}) (II).

Figure 3. Nine mesocosms placed in the refrigeration unit in the greenhouse. A measurement chamber is placed on top of the mesocosms during the gas flux measurements.

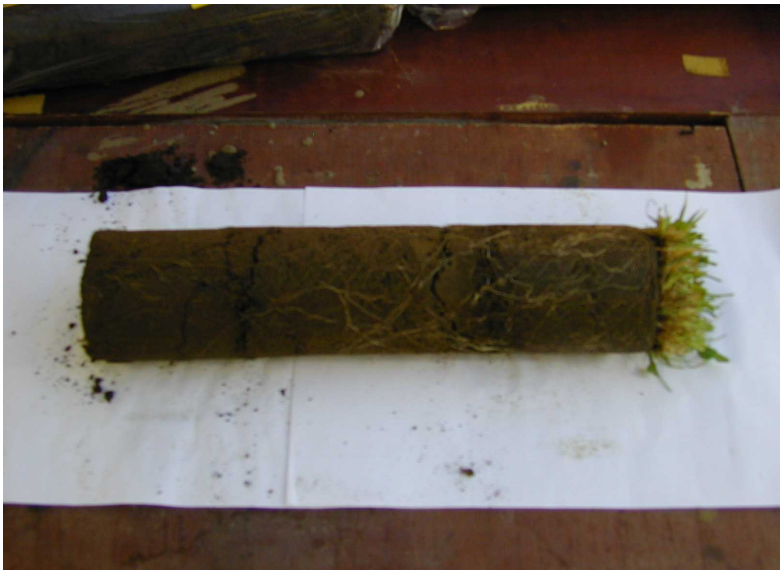

Figure 4. One sandy soil core after the experiment, including remaining biomass.

Table 1. Soil types, plant species, treatments, measured gases and biomass measurements of different studies.

	Studies			
	I	II	III	IV
Study soil	Peat	Sandy loam	Peat Sandy loam	Sandy loam
Plant species	*Phleum pratense*	*Phleum pratense*	*Phleum pratense*	*Phleum pratense* *Trifolium pratense*
Treatments	Two CO_2 levels: 360 and 720 ppm Different N levels 2, 6 and 10 g N m^{-2} Raised groundwater table combined with extra N application, 20 g N m^{-2}	Two CO_2 levels: 360 and 720 ppm Different N levels 5, 10 and 15 g N m^{-2} Raised groundwater table combined with extra N application, 20 g N m^{-2}	Two CO_2 levels: 360 and 720 ppm Two watering treatments: same watering and same moisture Same amount of N for all mesocosms, 10 g m^{-2} for peat and 15 g m^{-2} for sandy loam Raised groundwater table combined with extra N application, 20 g N m^{-2}	Two CO_2 levels: 360 and 720 ppm Different N levels 5 and 10 g N m^{-2} Raised groundwater table combined with extra N application, 20 g N m^{-2}
Measured gases	N_2O, CH_4	N_2O, CH_4, CO_2	N_2O, CH_4, CO_2	N_2O, CH_4
Biomass measurements	Harvest of above ground biomass five times Determination of root biomass Determination of N concentration of above-ground dry biomass	Harvest of above ground biomass four times Determination of root biomass Determination of N concentration of above-ground dry biomass	Harvest of above ground biomass four times Determination of root biomass Determination of N concentration of above-ground dry biomass	Harvest of above ground biomass four times Determination of *T. pratense* root nodules, thickness of the rootneck from main roots Determination of N concentration of above-ground dry biomass
Place of experiment	Greenhouse	Greenhouse Growth chamber	Growth chamber	Greenhouse
Duration of experiment	6 months	3.5 months	In peat soil 4.5 months In sandy soil 4.5 months	4 months

2.6.3 Potential CH_4 production and oxidation

At the end of the study I, the potentials for CH_4 production and oxidation in soil were measured. Before the peat samples were taken, the groundwater table in all the mesocosms was kept high to make sure that the lower part of the mesocosm soil profile remained anaerobic. To determine potential CH_4 production and oxidation, four 40 ml peat samples from all the 36 mesocosms were taken from depths of 5–10 cm and 35–40 cm. To determine CH_4 oxidation, two of

the peat samples were placed as a thin layer on the bottom of flasks, which were left to oxidise for several hours at room temperature. After sealing the flasks with a septum, the CH_4 concentration in the flasks was adjusted to ca. 100 ppm. CH_4 consumption was monitored, taking gas samples four or five times during an incubation period of 3 days. The oxidation rate was determined from the decrease in CH_4 concentration. To determine CH_4 production, the peat samples were placed in the flasks with deionised water. To maintain anoxic conditions, the flasks were sealed with a rubber septum and flushed with N_2 (99.96%). Potential CH_4 production was determined from the increase in the CH_4 content in the flask headspace during the 3 to 4 day incubation period at +15°C.

3 RESULTS AND DISCUSSION

3.1 *P. pratense* was acclimated to elevated atmospheric CO_2 concentration during the greenhouse experiment

The current atmospheric CO_2 concentration (ca. 380 ppm) is not the optimal concentration for photosynthesis since Rubisco is not CO_2-saturated at this concentration (Stitt 1991). Elevated atmospheric CO_2 concentration increased the NEE of the *P. pratense* cultivation system in peat soil with a high N supply (unpublished data, Fig. 5) and in the sandy soil (II, unpublished data) receiving low to high N. P_G was increased as was NEE under elevated CO_2. Enhanced photosynthesis is reported with grass species (e. g. Davey et al. 1999, Ainsworth et al. 2003, Ellsworth et al. 2004, Ainsworth and Long 2005). However, under elevated CO_2, photosynthesis has been found to acclimate, especially with low N supply due to sinklimitation, i.e. the development of sinks for photoassimilate is limited. Acclimation is defined as those physiological changes that occur when plants are grown under elevated CO_2 (e.g. Drake et al. 1997, Rogers et al. 1998).

The acclimation of *P. pratense* photosynthesis was seen in our studies by measuring the CO_2 exchange of mesocosms grown under elevated CO_2 in the ambient CO_2. P_G was lower than or as high as the P_G of mesocosms grown and measured at ambient CO_2 (II, unpublished data, fig 5), even if the biomass of 18 cm stubble was higher under elevated CO_2 (II, unpublished data). The higher photosynthetic rate under elevated CO_2 increases the amount of soluble carbohydrates in plant leaves, leading to a decrease in the rate of Rubisco carboxylation and thus a diminished amount of Rubisco (Drake et al. 1997, Ainsworth et al. 2003). The decreased N concentration in the above ground biomass of *P. pratense* in all studies (I–IV) was probably partly caused by the decrease in the amount of Rubisco (Drake et al. 1997, Davey et al. 1999).

The acclimation of *P. pratense* photosynthesis was expressed irrespective of the N fertilisation treatment and is in accordance with the study by Ainsworth et al. (2003), who found that acclimation can also take place with high N availability. Earlier studies (e.g. Rogers et al. 1998, Bryant et al. 1998) argued that acclimation occurred mainly with a low N supply or in conditions where growth may be sink-limited. However, in agricultural practice, the acclimation of grasses may be absent when sink-limitation is removed after harvesting the aboveground biomass, i.e. when canopy size is small (Rogers et al. 1998).

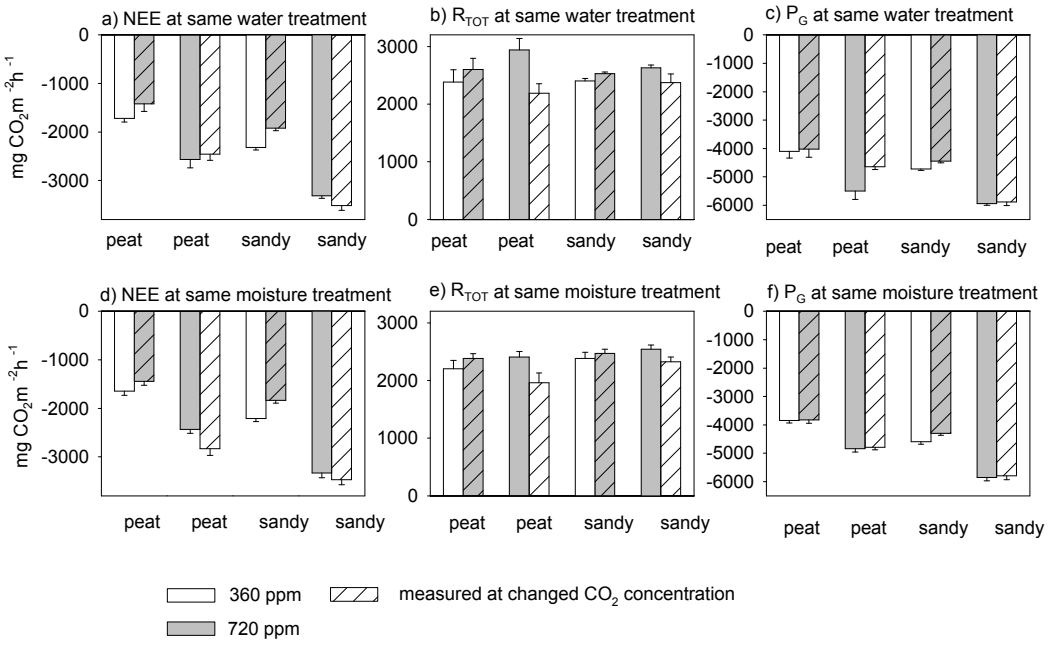

Figure 5. Unpublished data on instant CO_2 exchange (P_G, NEE and R_{TOT}) with two watering and CO_2 treatments using high N supply for peat and sandy soil grown *P. pratense*. Measurements were done at normal growing and changed CO_2 concentration, i.e. the CO_2 exchange of mesocosms grown under ambient CO_2 (360 ppm) was measured under elevated (720 ppm) CO_2 concentration and *vice versa*.

3.2 Elevated CO_2 affects total respiration rate of mesocosms

Elevated CO_2 concentration increased R_{TOT} with the same watering and the same moisture treatments at both soils with high N input (unpublished data, Fig. 5). The difference was significant (P = 0.002, 2-way ANOVA) only in the sandy soil (10% increase) with the same watering treatment. For the peat soil, the difference (23% increase) was only indicative (P = 0.078, 2-way ANOVA) (unpublished data, Fig. 5). However, with the sandy soil, the R_{TOT} was slightly decreased with high N and the same moisture treatment (II). The changes in R_{TOT} are probably consequence of changes in below ground respiration, as dark respiration of leaves has not found to be markedly affected (Tjoelker et al. 2001).

The R_{TOT} seems to be coupled to the biomass production of *P. pratense* and P_G under elevated CO_2. Soil respiration may be enhanced due to the increased supply of new C to the soil (Cotrufo and Gorissen 1997, Suter et al. 2002). Moreover, the assumption was that increased soil moisture under elevated CO_2 would affect the R_{TOT}. However, the increase in topsoil moisture was minor, at least in the peat soil, and no positive correlation between R_{TOT} and the topsoil moisture was found (unpublished data, Fig 6). Thus the increment in R_{TOT} was more likely caused by enhanced photosynthesis stimulated by elevated CO_2, which augments the carbohydrate supply to the roots, increasing root and rhizospheric respiration (Craine et al. 1999, Aeschlimann et al. 2005, Søe et al. 2004). Moreover, the enhanced biomass production of roots provides additional substrates for soil micro-organisms (Zak et al. 2000). Aeschlimann et al. (2005) found that

night-time ecosystem respiration was affected by midday NEE of the preceding day, suggesting that total respiration is linked to the availability of recently assimilated C. Consequently, in our experiments, the rate of R_{TOT} was the higher, the greater the increment in NEE and P_G was (Fig. 5 and II).

Further, a study by Jastrow et al. (2005) argued that more C can accumulate in the mineral soil under elevated CO_2 concentration due to increased root production. The findings of study II and unpublished data on increased production of residual biomass might indicate enhanced C accumulation in the agricultural mineral soil under elevated CO_2 concentration.

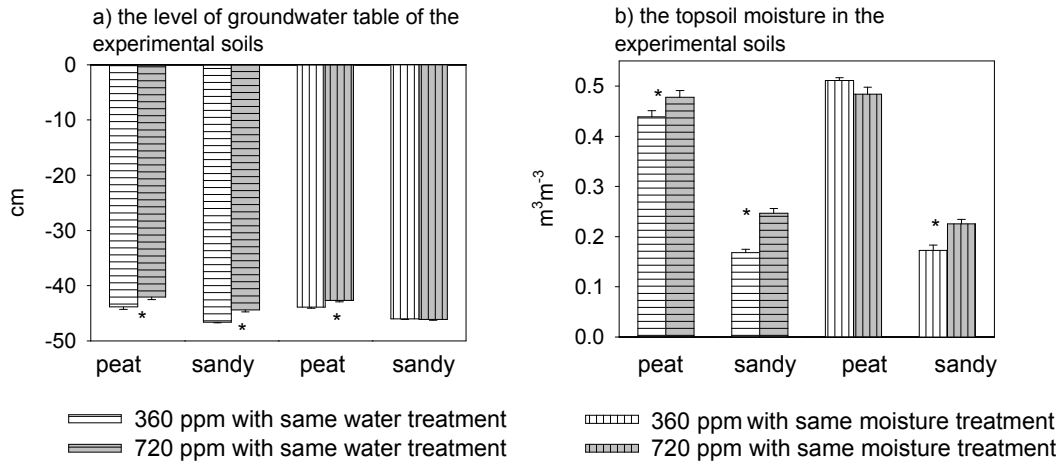

Figure 6. Unpublished data on topsoil moisture content (m^3m^{-3}) and the level of groundwater table (cm) of peat and sandy soils during the instant CO_2 exchange measurements at two watering and CO_2 treatments. The asterisk indicates a statistical difference between the CO_2 treatments ($P < 0.05$, Mann-Whitney Test)

3.3 Biomass production of *P. pratense* and *T. pratense* was increased under elevated CO_2 concentration

Agricultural biomass production should be divided into harvestable biomass, i.e. yield production, and remaining biomass (residual biomass or non-harvested biomass), including stubble, aftermath and roots. Above ground biomass consists of harvested biomass and remaining biomass.

3.3.1 Harvestable biomass production

Elevated CO_2 concentration increased the yield of *P. pratense* and the mixed stand of *Pratense/Trifolium* on the sandy soil, even with the lowest N fertilisation level (II, IV, Table 2). With the peat soil, the increase in yields of *P. pratense* at elevated CO_2 required more fertiliser N (I, III, Table 2) than with the sandy soil.

Table 2. Summarised results of four studies on above and below ground biomass production in peat soil: yields, stubble, roots, shoots, total N concentration and the amount of harvested N with different N and watering treatments under elevated and ambient CO_2.

	Peat soil			
Phleum pratense (I, III)				
	same moisture (I, III)			same water (III)
Yields	low N (I)	moderate N (I)	high N (I, III)	high N
1st	ns	↑	↑ (I, III)	↑
2nd	ns	ns	ns(I) ↑ (III)	↑
3rd	ns	ns	ns(I) ↑ (III)	ns
4th (III)			ns	ns
Stubble	ns	ns	↑ (I)	↑
Roots	ns	↑	↑ (I, III)	ns
Total biomass	ns	↑	↑ (I, III)	↑
Shoots	ns	ns	↑ (III)	ns
N%				
1st harvest	↓	↓	↓ (I, III)	ns
2nd harvest	↓	↓	↓ (I)	
3rd harvest	↓	↓	↓ (I)	
4th harvest (III)			↓	ns
N g m^{-2}				
1st harvest	ns	ns	ns (I)	ns
2nd harvest	↓	ns	ns (I)	
3rd harvest	↓	ns	↓ (I)	
4th harvest (III)			ns	ns

ns = statistically no significant effect

↓ = decrease

↑ = increase

Table 3. Summarised results of four studies on above and below ground biomass production in sandy soil: yields, stubble, roots, shoots, total N concentration and the amount of harvested N with different N and watering treatments under elevated and ambient CO_2.

	Sandy loam soil					
Phleum pratense (II-IV)					*Trifolium pratense* (IV)	
	same moisture (II-IV)			same water (III)	same moisture	
Yields	low N (II, IV)	moderate N (II, IV)	high N (II, III))	high N	low N	moderate N
1st	ns (II)↑ (IV)	ns (II)↑ (IV)	ns(II)↑ (III)	↑	↑	ns
2nd	↑ (II, IV)	↑ (II, IV)	↑ (II, III)	↑	↑	ns
3rd	↑ (II, IV)	↑ (II), ns (IV)	↑ (II, III)	↑	ns	ns
4th (III)			↑	ns		
Stubble	ns (II, IV)	↑ (II), ns (IV)	ns (II)↑ (III)	ns	ns	ns
Roots	↑ (II)	↑ (II)	↑ (II, III)	↑	nd	nd
Total biomass	↑ (II)	↑ (II)	↑ (II, III)	↑	nd	nd
Shoots	↑ (II)	↑ (II)	↑ (II, IV)	ns	ns	ns
N%						
1st harvest	↓ (II), ns(IV)	↓ (II, IV)	↓ (II, III)	↓	ns	↓
2nd harvest	↓ (II, IV)	↓ (II, IV)	ns (II)↓ (III)		ns	↓
4th harvest (III)			↓	↓		
N g m^{-2}						
1st harvest	↓ (II)↑ (IV)	↓ (II), ns (IV)	↓ (II, III)	↓	ns	ns
2nd harvest	ns (II, IV)	ns (II, IV)	ns (II, III)		ns	↑
4th harvest (III)			ns	ns		

nd = not determined

ns = statistically no significant effect

↓ = decrease

↑ = increase

Several studies have shown an increase in the biomass production of grassland species with elevated CO_2, especially with *Lolium perenne* and *Trifolium repens* (e.g. Sage et al. 1989, Drake et al. 1997, Cardon et al. 2001, Elssworth et al. 2004).

It was not expected that elevated CO_2 would enhance the yield of *T. pratense* similarly to that of *P. pratense* (IV). One assumption was that the harvestable biomass production under elevated CO_2 concentration would be higher with *T. pratense*. Hebeisen et al. (1997) found that in bi-species mixed grass cultivation, *T. repens* markedly increased the yields under elevated CO_2, while the yields of *L. perenne* decreased. Ainsworth and Long (2004) concluded that legumes produce more biomass than C_3 grasses

under elevated CO_2. Sæbø and Mortensen (1995) showed that *T. pratense* increased its dry weight production by 30% with elevated CO_2. Legumes are able to fix N_2, and biomass production with an enhanced supply of CO_2 is not restricted by N availability (Zanetti et al. 1997). Perhaps some of this fixed N_2 was utilized by *P. pratense* (Boller and Nösberger 1987, Ledgard and Steele 1992) resulting enhanced yield production under elevated CO_2.

3.3.2 Elevated CO_2 concentration decreased total N concentration in the above ground biomass but increased the yield of N with a mixed stand

The total N concentration in the above ground biomass decreased under elevated CO_2 concentration, as did the amount of harvested N (Table 2 and 3). A decrease in the N concentration of the above ground dry matter is well documented (e.g. Cotrufo et al. 1998, Hartwig et al. 2000). This decrease may be a consequence of decreased investment in Rubisco (Stitt 1991, Davey et al. 1999) and/or dilution (carbohydrates accumulate in leaves) (Fischer et al. 1997) or increased N allocation to root biomass (van Ginkel et al. 1997, Cotrufo et al. 1998). A decrease in N concentration can lower the N yield of above ground biomass (Zanetti et al. 1997, Gloser et al. 2000). However, with the sandy soil, the N yield of *P. pratense* increased under elevated CO_2 concentration with the low N treatment, when it was cultivated together with *T. pratense* (IV). This probably implies that in the mixture of *Phleum/Trifolium*, the availability of N for use is ameliorated. The N concentration of *T. pratense* decreased with the moderate N treatment in contrast to the N yield (Table 2, IV). The N_2 fixation capacity of *T. pratense*, which is known to increase under elevated CO_2 concentration (Zanetti et al. 1996), favours N availability for biomass production in the mixture of *Phleum/Trifolium*.

3.3.3 Remaining biomass of P. pratense *was increased under elevated CO_2 concentration*

The yield is a part of the produced biomass, and does not reflect the total biomass production at elevated CO_2 concentrations. During recent years, more attention has been paid to non-harvested biomass production, which increases markedly under elevated CO_2 (Daepp et al. 2001, Schneider et al. 2006). In our experiments, the stubble of *P. pratense*, including aftermath, was increased under the elevated CO_2 treatment with a high N fertilisation level in peat soil and with the moderate and high N treatments in sandy soil (Table 2 and 3). The increment could be caused partly by the enhanced branching of shoots under elevated CO_2, which is typical for *P. pratense* (Mortensen and Sæbø 1996).

The root production of *P. pratense* increased under elevated CO_2 concentration in both experiment soils with all treatments, although in the peat soil the difference was not statistically significant with the lowest N treatment and the same water treatment (Table 2 and 3). An increment in root production was evident, especially in the upper layers of the soil (unpublished data). Approximately 75 – 88% of the total root biomass of *P. pratense* was located in the upper 20 cm of the soil, which is in agreement with Bolinder et al. (2002) and Crush et al. (2005). The increase in the root production of grasses due to elevated CO_2 is well documented (e.g. Ryle et al. 1992, Hebeisen et al. 1997, Gorissen and Cotrufo 2000, Jastrow et al. 2000, Cardon et al. 2001, Suter et al. 2002, Phillips et al. 2006, Hill et al. 2007). The thickness of the *T. pratense* main root and the observed amount of root nodules was not found to be change under elevated CO_2 in contrast with the ambient CO_2 concentration.

Increased root production is the main pathway by which more new C is supplied to the soil under elevated CO_2 concentrations (e.g. van Ginkel and Gorissen 1998, Gorissen and Cotrufo 2000, Niklaus et al. 2001,

Jastrow et al. 2005). This may lead to enhanced C accumulation in agricultural soil (Jastrow et al. 2005, Hill et al. 2007). Elevated CO_2 can, however, enhance overall C cycling more than C sequestration in the soil (Hungate et al. 1997), thus increasing the rapidly cycling C pools in soil. These pools are roots (exudation and turnover) (Hungate et al. 1997), surface detritus (Niklaus et al. 2001), soil micro-organisms (Cotrufo and Gorissen 1997, Hungate et al. 1997, Hu et al. 2001) and rhizodeposition (Pendall et al. 2004).

3.4 N_2O fluxes

3.4.1 Before the first harvest elevated CO_2 tended to decrease the N_2O fluxes

The high N_2O fluxes occurred at the beginning of the experiments, i.e. before the first harvest, probably due to the minor N consumption of plants during the early stage of growth. The N_2O fluxes tended to be higher under ambient CO_2 concentration before the first harvest (I–IV). After germination, the plants grew faster under elevated CO_2, consuming more N compared with growth at ambient CO_2 concentration (I, III and IV), resulting in higher yield production. At the beginning of the experiment, part of the N_2O was most likely produced by nitrification due to the low groundwater table. Nitrification may be a dominant process for N_2O in dry soils (Davidson 1991, Pihlatie et al. 2004). Furthermore, the nitrification rate is known to increase as a result of disturbances, such as fertilisation (Schlesinger 1997). The start of the experiment, including the sieving of the soil, can be comprehended as a disturbance, and these disturbances could partly explain the high N_2O fluxes at the beginning of the experiments.

3.4.2 Elevated CO_2 concentration increases N_2O fluxes after the harvest of P. pratense *above ground biomass if water and nitrogen are adequately available*

Elevated CO_2 concentration increased N_2O emissions under *P. pratense* from the peat and sandy soils. The increase generally occurred as a short burst immediately after the harvest, followed by N fertilisation and watering (I, III). Several studies have reported higher N_2O emission rates under elevated CO_2 from agricultural soils (e.g. Arnone and Bohlen 1998, Ineson et al. 1998, Baggs and Blum 2004), and especially after N fertilisation combined with an increase in soil moisture (Ineson et al. 1998). It is argued that enhanced root-derived C fuels denitrification, producing N_2O.

The N_2O fluxes from the sandy soil were higher under elevated CO_2 concentration with the high N treatment, not only immediately after the harvest combined with N fertilisation, but during the whole experiment (III). However, when the groundwater table was very low (ca. – 46 cm) the N_2O fluxes did not increase under elevated CO_2 (II). Thus, elevated CO_2 concentration increases N_2O fluxes if the soil moisture is high enough to support denitrification.

3.4.3 Elevated CO_2 either decreased or increased N_2O fluxes with a raised level of groundwater table and excess N availability

A raised groundwater table combined with extra NPK fertilisation had no clear effect on N_2O fluxes under elevated CO_2 in contrast to ambient CO_2 (I–IV). Enhanced C accumulation in the soil is assumed to enhance N_2O emissions under elevated CO_2 resulting from an increase in denitrification (Baggs et al. 2003), if anaerobiosis is high enough. N_2O is produced in several microbial processes (autotrophic and heterotrophic nitrification, denitrification, aerobic denitrification, coupled nitrification and denitrification), which could occur concurrently and adjacently, de-

pending on soil conditions, especially soil water (Pihlatie et al. 2004, Bateman and Baggs 2005). In addition, N_2O is an intermediate regime in denitrification and can be reduced to N_2 (Firestone and Davidson 1989). However, in the peat soil, when the groundwater table was very high, the N_2O emission was lower under elevated than under ambient CO_2 concentrations (III). At high soil moisture, N_2 would be dominant in the end products of denitrification.

The highest N_2O emissions took place under elevated CO_2 with the lowest N fertilisation treatment supplied by extra NPK fertilisation combined with the harvest and raised groundwater table (I, II, IV). The plants (*P. pratense* and a mixed stand of *Phleum*/*Trifolium*) growing with the low N supply were probably adapted to the low nutrient availability, and when the nutrient availability was suddenly enhanced, the plants were not able to utilise the excess NPK. Hence, under elevated CO_2, more nutrients were available for microbial use associated with enhanced C supply (Cotrufo and Gorissen 1997, Jastrow et al. 2000). This promotes microbial processes such as denitrification, enhancing N_2O fluxes (I, II, IV) (Baggs et al. 2003).

Consequently, it is not possible to argue that the higher N_2O emissions under elevated CO_2 resulted from an increase in soil moisture due to enhanced water use efficiency of *P. pratense*, as this would require a more drastic water increase in soil.

3.4.4 Elevated CO_2 did not increase the N_2O emissions from a mixed stand of T. pratense/P. pratense *grown in sandy soil*

The N_2O emissions from sandy soil with a mixed stand of *Trifolium/Phleum* decreased under elevated CO_2 concentration unless groundwater table was raised and extra inorganic N was added (IV). It is suggested that the presence of a N_2-fixing legume increases N_2O fluxes under elevated CO_2 due to the increased C and N supply to the soil (Hungate et al. 1997, Niklaus et al. 2001, Ainsworth et al. 2003). However, there was no such burst in N_2O emissions under elevated CO_2 after the harvest followed by N fertilisation combined with the watering as there was from the mesocosms on sandy soil under *P. pratense* (II, III).

The mixed stand of *Trifolium/Phleum* produced more biomass with low and moderate N treatments, but did not generate higher N_2O emissions. On the contrary, the N_2O emissions were diminished (IV). Pihlatie et al. (2004) found that nitrification is the main process contributing to N_2O emissions from sandy loam soil, even with high WFPS%. In addition, Niklaus et al. (2006) argued that potential nitrification rates increase in the presence of legumes because net N mineralisation exceeds the demand of the plants, leaving more NH_4^+ for nitrifiers. As nitrification does not benefit enhanced new C, N_2O emissions will not increase with elevated CO_2 concentration until there are favourable conditions for denitrification. With extra N addition combined with a raised groundwater table, N_2O fluxes were higher under elevated CO_2. Nitrification was most likely the predominant process with a low groundwater table. In dry soil, NO_3^- could have been accumulated and after watering, there could have been increase in denitrification (Abbasi and Adams 2000). Because mesocosms are closed systems with no leaching, NO_3^- availability was probably increased.

3.5 CH_4 fluxes

There were no changes in the CH_4 fluxes in peat soil under elevated CO_2 concentration. Even with a raised groundwater table, CH_4 emissions did not emerge (I). Both soils had negligible CH_4 fluxes with all treatments (I, unpublished data). There was no change in CH_4 oxidation or production potentials in the peat soil under elevated CO_2 concentration (I). Due to the increase in soil moisture (Drake et al. 1997, Niklaus et al. 1998) and C supply (Cotrufo and Gorissen 1997, Suter

et al. 2002), the emissions of CH_4 could have been increased under the elevated CO_2.

Agricultural soils are generally of minor importance as a sink for atmospheric CH_4 (Conrad 1989, Flessa 1998, Maljanen et al. 2003a) or source of CH_4 (Nykänen 1998). Here the negligible production of CH_4 in peat soil can be explained by the low amount of methane-producing archae in the drained peat (Willison et al. 1998). When the soil oxygen content was reduced by increasing soil moisture, the methane-producing archae require a long time to recover (Kettunen et al. 1999). CH_4 uptake was also negligible during the experiment. The low rate of oxidation might be a result of soil dryness due to drainage or of soil drying during storage, thus causing stress for the methane oxidisers (Dobbie and Smith 1996). Both sandy and peat soils consumed CH_4 in situ at a rate of –800 to –1200 g CH_4 $ha^{-1}a^{-1}$ and –530 to –106 g CH_4 ha^{-1} a^{-1}, respectively (Martikainen et al. 2002).

4 CONCLUSIONS

The following conclusions can be drawn:

1. Elevated CO_2 concentration increases N_2O fluxes from agricultural peat and sandy soil under *P. pratense,* but it requires simultaneous watering or a raised groundwater table and N availability.
2. CH_4 dynamics is not affected by elevated CO_2 concentration.
3. Biomass yield increases under elevated CO_2 with a low to high N supply in sandy soil. In peat, yields increase with moderate and high N. *P. pratense* increases root production under elevated CO_2 concentration.
4. N concentration in the above ground biomass of *P. pratense* and *T. pratense* decreases under elevated CO_2, lowering N yield. By contrast, the presence of legume *T. pratense* in the mixture increases the N yield under elevated CO_2.
5. Soil moisture increases under elevated CO_2, but the effects on N_2O, CH_4 and CO_2 fluxes are not obvious.
6. Photosynthesis of *P. pratense* acclimates to a higher supply of atmospheric CO_2 irrespective of N fertilisation treatment.
7. Elevated CO_2 does not increase N_2O fluxes from sandy soil under a mixed stand of *Trifolium/Phleum*, unless there is excess N availability and a high groundwater table.

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