Can a mixed stand of N₂-fixing and non-fixing plants restrict N₂O emissions with increasing CO₂ concentration?

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Abstract

Initial effects of elevated atmospheric CO₂ concentration on N₂O fluxes and biomass production of timothy/red clover were studied in the laboratory. The experimental design consisted of two levels of atmospheric CO₂ (ca. 360 and 720 μmol CO₂ mol⁻¹) and two N fertilisation levels (5 and 10 g N m⁻²). There was a total of 36 mesocosms comprising sandy loam soil, which were equally distributed in four thermo-controlled greenhouses. In two of the greenhouses, the CO₂ concentration was kept at ambient concentration and in the other two at doubled concentration. Forage was harvested and the plants fertilised three times during the basic experiment, followed by harvest, a fertilisation with the double amount of nitrogen and rise of water level. Under elevated CO₂, harvestable and total aboveground dry biomass production of a mixed *Trifolium/Phleum* stand was increased at both N treatments compared to ambient CO₂. The N₂O flux rates under ambient CO₂ were significantly higher at both N treatments during the early growth of mixed *Phleum/Trifolium* mesocosms compared to the N₂O flux rate under elevated CO₂. However, when the conditions were favourable for denitrification at the end of the experiment, i.e. N availability and soil moisture were high enough, the elevated CO₂ concentration enhanced the N₂O efflux.

1. Introduction

The current atmospheric CO₂ concentration (ca. 380 μmol CO₂ mol⁻¹, Mauna Loa) is almost 100 μmol CO₂ mol⁻¹ above the pre-industrial average, and it is still rising due to human activities like the combustion of fossil fuels and deforestation (Houghton et al., 2001). The concentration of other greenhouse gases, like N₂O, is also increasing. In addition to absorbing heat radiation, N₂O destroys stratospheric ozone (Houghton et al., 2001). The concentration of other greenhouse gases, like N₂O, is also increasing. In addition to absorbing heat radiation, N₂O destroys stratospheric ozone (Houghton et al., 2001). Agriculture is one of the main anthropogenic sources of N₂O, accounting e.g. in Finland for almost half of the national N₂O emissions (Pipatti, 1997). N₂O originates from microbial N cycling in soil, mostly from aerobic nitrification or from anaerobic denitrification (Firestone and Davidson, 1989). High emissions from agricultural soils are associated with a high N availability in the soil, resulting from the addition of fertiliser N and biological N₂ fixation (Houghton et al., 2001).

An increased atmospheric CO₂ concentration is known to increase C flow into the soil via enhanced photosynthesis and, as a consequence, increased biomass production (e.g. Ryle et al., 1992; Niklaus et al., 2001; Suter et al., 2002; Ainsworth et al., 2003). Legumes are able to fix N₂ biologically and their N₂ fixation has been reported to be enhanced due to an increased supply of atmospheric CO₂ (Zanetti et al., 1996; Lee et al., 2003). These CO₂-induced increases in C and N input in the soil increase substrate availability for denitrifying bacteria and may result in higher N₂O emission from agricultural soils in the future. It is now known, however, that the magnitude of N₂O emissions depends on the soil type (e.g. Velthof and Oenema, 1995; Maag and Vinther, 1996; Pihlatie et al., 2004; Syväsalo et al., 2004), plant species (e.g. Hénault et al., 1998; Syväsalo et al., 2004; Niklaus et al., 2006), soil
temperature (Maag and Vinther, 1996; Gödde and Conrad, 1999; Koponen et al., 2006), moisture content (Maag and Vinther, 1996; Phlathie et al., 2004; Del Prado et al., 2006) and type of fertiliser (Ellis et al., 1998). N$_2$O emissions from agricultural fields under elevated CO$_2$ concentration with different N fertilisation doses have been studied over a period of several years, especially in the FACE studies at Eschikon, Switzerland (Ineson et al., 1998; Baggs et al., 2003b). Emissions of N$_2$O have been reported to increase under elevated CO$_2$ concentration after the application of inorganic N fertiliser (Ineson et al., 1998; Kettunen et al., 2005), especially with increased soil moisture content (Abbasi and Adams, 2000) and a high groundwater level (Kettunen et al., 2005, 2006).

The aim of our greenhouse experiment was to determine how an increased supply of atmospheric CO$_2$ and different N doses affect N$_2$O fluxes from farmed sandy soil growing Phleum pratense (timothy) and Trifolium pratense (red clover). In addition, we determined the aboveground biomass production of P. pratense and T. pratense, the amount of harvested N, and the amount of root nodules on T. pratense roots. The hypotheses were that: (1) an elevated CO$_2$ concentration would increase N$_2$O fluxes from sandy soil under mixed Phleum/Trifolium, and (2) a high N availability combined with a high groundwater table would enhance this increase in fluxes under elevated CO$_2$. P. pratense and T. pratense were selected because P. pratense is a major grass and forage crop species in Finland, and T. pratense is the legume most used in mixed stands. Both species are widely used in the boreal zone.

2. Materials and methods

2.1. Characteristics of the experimental soil

The sandy (sandy loam, FAO classification: Eutric Cambisol) soil for the experiment was taken from an agricultural field at Jokioinen (60°49'N, 23°30'E), southern Finland, in June 2002. The field had been used for conventional agriculture for decades. The soil was kept at +5°C for ca. 1 month before the experiment. The sandy soil contained 0.16% w/w N and 2.4% w/w organic C, the C:N ratio was 15, pH (H$_2$O) 6.0 and bulk density 1.2 g cm$^{-3}$.

2.2. Experimental set-up

In July 2002, the sandy soil was sieved (mesh size 2 mm) and 4.7 kg soil was placed in each of the 36 mesocosms and irrigated with 420 ml deionised water. Each mesocosm consisted of a PVC tube (height 47 cm, diameter 10 cm) that was closed at the bottom with a plastic plug. The mesocosms were randomly distributed in four greenhouses (ca. 2.67 m$^3$), each greenhouse containing nine mesocosms. In two greenhouses, the CO$_2$ concentration was kept close to the ambient (360 μmol mol$^{-1}$), and in the other two it was doubled (720 μmol mol$^{-1}$). Each greenhouse had a refrigerator unit that cooled the bottommost 40 cm of the mesocosms. The top 7 cm of each mesocosm was above the refrigerator unit. The temperature in the greenhouses and refrigerator units was set at +20 and +15°C, respectively.

After 4 days of adaptation to greenhouse conditions, the mesocosms were sowed. P. pratense (2.48 g m$^{-2}$) and T. pratense (1.52 g m$^{-2}$) seeds were mixed with ca. 30 g sifted soil and spread onto the mesocosms, which were then watered with 50 ml of deionised water. Fifteen days after sowing, when the seeds had already germinated, fertiliser was applied onto the mesocosms, which were then watered with 100 ml of deionised water. Mesocosms received either low (5 g m$^{-2}$) or moderate (10 g m$^{-2}$) N fertilisation, i.e. there were nine replicates of each fertilisation level in both CO$_2$ treatments. The fertiliser used was Yields N-P-K 26-2-3 fertiliser (N = 26%, P = 2%, K = 3% w/w, Kemira Agro Oy, Finland) in which N was given as NH$_4$NO$_3$. Five weeks after sowing, the P. pratense and T. pratense were thinned, leaving 12 shoots of P. pratense and 3 shoots of T. pratense per mesocosm. The experiment continued for 4 months, which almost corresponded to the thermal growing period in Finland, which is on the average 150 days. The experiment was divided into four measurement periods with combined harvesting of growth and fertilisation.

During the basic experiment (up to day 290), the low and moderate fertilisation treatments received a total of 15 and 30 g N m$^{-2}$, respectively, due to fertilisation in the beginning and following each harvest (see Fig. 2). The low and moderate fertilisation treatments correspond to the normal management practice in grass and silage production on sandy soils in Finland. After the basic experiment, extra fertilisation of 20 g N m$^{-2}$ was applied to all the mesocosms in order to determine the potential effect of increased C supply without N limitation on the N$_2$O efflux. The level of the groundwater table was raised (see Table 1) in connection with the extra NPK fertilisation in order to provide suitable conditions for the denitrification process. Due to the malfunction of the gas chromatograph we lost the fluxes following the first extra fertilisation, and therefore we had to renew the extra fertilisation treatment combined with harvest 3 weeks after the previous harvest and extra fertilisation.

2.3. Measurements of N$_2$O fluxes

Gas sampling for determination of the N$_2$O fluxes was started 15 days after sowing and 1 day after fertilisation. Gas samples were taken using a closed vented opaque chamber (ca. 3.5 dm$^3$) technique and analysed using a gas chromatograph equipped with an electron capture detector within 16 h. The samples were taken every 5 min during a 15-min sampling period. The first sample was taken immediately after the chamber was placed on the water-filled groove around the top of the mesocosm so that the interface between the two was airtight. Inside the chamber there was a small blower to mix the air in the chamber. N$_2$O fluxes from each of the 36 mesocosms were measured once a week, and for 2 weeks after harvest and fertilisation.
twice a week. The flux rates of the mesocosms were calculated from the linear change in gas concentration in the chamber.

2.4. Vegetation measurements

The aboveground plant material was harvested four times during the experiment by cutting the stand of *P. pratense* and *T. pratense* to a height of 5 cm (see Fig. 2). The dry weight of the clipped material was determined by drying the fresh material at +40 °C for 2 weeks. Before drying the biomass, the harvested biomass of *P. pratense* and *T. pratense* was separated from each other according to the species. Total N% was determined on the first and second harvests by the Kjeldahl method (Vapodest 30, Gerhardt, Germany). At the end of the experiment, the number of living branched shoots of *P. pratense* and *T. pratense* in each mesocosm was counted. The sandy soil from the mesocosms was stored in a freezer at −20 °C for over 2 years. In the spring of 2005 the remaining aboveground biomass (stubble) and main roots of *T. pratense* were subsequently separated from the thawed soil. The remaining stubble of *T. pratense* and *P. pratense* was separated according to the species, and the dry weight was determined as described above. The thickness of the root neck on the main roots of *T. pratense* was measured and the number of visible root nodules counted.

2.5. Controlling the growing conditions

Air temperature, irradiation and CO₂ concentration in the greenhouses were recorded automatically every 5 s with an intelligent sensor module (Gantner ISM 112, Austria), which transmitted all the data to a computer via an integrated RS485 communication interface. The CO₂ concentration in the greenhouses was measured with an infrared detection system (GMP 111 CO₂ Transmitter, Vaisala) and, when necessary, the flow of CO₂ from a pressure tank to the greenhouse was automatically adjusted. For a more detailed description see Kettunen et al. (2005). Once a week every set (one set contained nine mesocosms) in the refrigerator unit was moved 90° clockwise so that three different mesocosms were placed against the back wall of the greenhouse. The location of the individual mesocosms was also changed so that each of the nine mesocosms was in the middle of the set for only 1 week at a time. At 4 weeks’ interval each set was moved to another greenhouse and refrigerator unit. After the move, the CO₂ concentration in the greenhouses was adjusted to correspond to the growth conditions of the particular set. The CO₂ concentration in the greenhouses was adjusted during 40–50 min. This was done in order to ensure that every mesocosm had the same growing conditions during the experiment.

For monitoring and controlling the groundwater table, a thin plastic perforated tube (diameter 2 cm, length 50 cm) with small holes at 5 cm intervals was inserted in each mesocosm. In order to maintain equal moisture conditions in each mesocosm the depth of the groundwater table in the mesocosms was monitored 5–6 times a week using a wooden stick with a scale. Part of the irrigation water was added via the perforated tube at the bottom of the mesocosm, and the rest was added to the top of the soil. In addition, 200 ml of deionised water was added to the mesocosms after each harvest followed by fertilisation to dissolve the fertiliser granules. Water addition was always applied about 17–24 h before the gas fluxes were measured. After the gas sampling, the moisture content (m^3 m^-3) of the soil was measured in the top of the mesocosm (6 cm) with a type ML2 Theta Probe connected to a type HH1 Theta Meter (Delta—T Devices, Cambridge, UK).

Soil temperature in the mesocosm was monitored with manual measurements via thermo-sensors, which were installed in two of the nine mesocosms in the same greenhouse. One had thermo-sensors at depths of 30, 20, 10 and 3 cm, and the other at depths of 30 and 3 cm. The soil temperature from thermo-sensors was measured (Fluke 52 k/J digital thermometer) when the gas samples were taken.

During the entire experiment natural light was supplemented by six metal halogen lights (400 W). For a more detailed description of the experimental set-up, mesocosms, fluxes and controlling the growing conditions, see Kettunen et al. (2005, 2006).

2.6. Statistical analyses

Normality of the distributions of the N₂O fluxes, dry aboveground biomass, number of living branched shoots, root nodules and thickness of the root neck of *T. pratense*, as well as total N% and g N m⁻² in the aboveground dry biomass, were tested using the Kolmogorov–Smirnov test. The homogeneity of the variances was tested using Levene statistics. Since the variances of the N₂O fluxes differed significantly, the difference in gas fluxes between the CO₂ treatments and between the N treatments was tested using a non-parametric Mann–Whitney U-test. Differences between the CO₂ treatments and between the N treatments in the aboveground biomass, number of living branched shoots, root nodules and thickness of the root neck of *T. pratense*, total N% and g N m⁻², as well as the moisture content in the topsoil (m³ m⁻³) and the height of the groundwater table during different measurement periods, were tested using a two-way ANOVA. Log transformation was carried out, when necessary. All the statistical analyses were performed using SPSS 13.0 for Windows statistical package.

3. Results

3.1. Growing conditions

There were no significant differences in air temperature, light intensity, soil temperatures, topsoil moisture content
or level of groundwater table between CO₂ treatments (Table 1).

3.2. Biomass

Elevated CO₂ significantly increased the total aboveground (three harvests of the aboveground biomass + stubble) and harvestable (three harvests) biomass of the mixed mesocosms (P. pratense and T. pratense) at both N treatments (Table 2). When the aboveground biomass production is considered by species, the harvestable biomass production of P. pratense increased under elevated CO₂ by 21% and 26% at low and moderate N treatments, respectively. Corresponding values for T. pratense were 22% and 18%. The total aboveground biomass of P. pratense increased under elevated CO₂ by 16% and 14% at low and moderate N treatments, respectively. Corresponding values for T. pratense were 21% and 18%.

The N concentration in the dry biomass of P. pratense and T. pratense decreased significantly under elevated CO₂ concentrations in the first and the second harvest at the moderate N level compared with the ambient CO₂. At the low N level, on the other hand, there was a difference in the N concentration of the P. pratense dry biomass only in the second harvest (Fig. 1). In contrast, the amount of harvested N (g N in dry matter m⁻²) in the harvestable dry biomass of P. pratense increased due to the elevated CO₂ concentration. The harvested amount of N in the dry matter yield was twice as much as the N applied in the low N treatment. With moderate N, still more N was harvested than had been applied (Fig. 1).

The CO₂ and N fertilisation treatments had no impact on the thickness of the root neck or the number of visible root nodules in the main roots of T. pratense or on the number of living branched shoots of T. pratense and P. pratense.

3.3. N₂O fluxes

The N₂O fluxes varied during the experiment depending on the time of harvest, together with N fertilisation and watering (Fig. 2). After emergence of the mixed stand and during vegetative growth before the first harvest and N fertilisation, the N₂O fluxes were significantly higher under ambient CO₂ at low (P = 0.001, ca. 55%) and at the moderate (P<0.0005, ca. 72%) N treatments. After the first harvest, the average N₂O fluxes of the measurement periods declined substantially, especially under ambient CO₂, from ca. 1212 to ca. 161 μg N₂O m⁻² h⁻¹ and from 850 to 154 μg N₂O m⁻² h⁻¹ at the moderate and low N

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**Table 1**

Conditions in the greenhouses, soil temperature at different depths and topsoil moisture content and level of groundwater table during the experiment

<table>
<thead>
<tr>
<th>Greenhouse</th>
<th>360 μmol CO₂ mol⁻¹</th>
<th>720 μmol CO₂ mol⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂ (μmol mol⁻¹)</td>
<td>369 ± 26</td>
<td>713 ± 35</td>
</tr>
<tr>
<td>Light (μM m⁻² s⁻¹)</td>
<td>350 ± 140</td>
<td>361 ± 149</td>
</tr>
<tr>
<td>Soil temperature (°C) depth 3 cm</td>
<td>19.6 ± 0.9</td>
<td>19.5 ± 0.7</td>
</tr>
<tr>
<td>10 cm</td>
<td>17.4 ± 1.5</td>
<td>17.3 ± 1.2</td>
</tr>
<tr>
<td>20 cm</td>
<td>17.2 ± 1.2</td>
<td>17.2 ± 0.9</td>
</tr>
<tr>
<td>30 cm</td>
<td>16.3 ± 0.8</td>
<td>16.3 ± 0.8</td>
</tr>
<tr>
<td>Topsoil moisture, (m³ m⁻²)/level of groundwater table (cm) in the beginning = julian day 203</td>
<td>0.224 ± 0.031/0</td>
<td>0.193 ± 0.022/0</td>
</tr>
<tr>
<td>during the basic exp. = julian days 218–290</td>
<td>0.200 ± 0.053/1.0 ± 1.75</td>
<td>0.215 ± 0.050/1.0 ± 1.61</td>
</tr>
<tr>
<td>in the last period = julian days 316–339</td>
<td>0.342 ± 0.054/16.5 ± 13.0</td>
<td>0.337 ± 0.051/14.5 ± 12.7</td>
</tr>
</tbody>
</table>

Mean ± S.D. is shown. Light (μM m⁻² s⁻¹) intensity is calculated as the average of 16 h light period in the greenhouses. Level of groundwater table, cm = the amount of water from the bottom of mesocosms. In the last period = after lifted level of groundwater table.

**Table 2**

The effect of CO₂ at two N fertilisation treatments on the aboveground biomass in three harvests, stubble and total aboveground biomass

<table>
<thead>
<tr>
<th>360 μmol CO₂ mol⁻¹/720 μmol CO₂ mol⁻¹</th>
<th>Both species</th>
<th>P. pratense</th>
<th>T. pratense</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 g N m⁻²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First harvest</td>
<td>339 ± 12.3/432 ± 18.4*</td>
<td>200 ± 6.56/262 ± 17.7*</td>
<td>139 ± 9.33/170 ± 10.2*</td>
</tr>
<tr>
<td>Stubble</td>
<td>361 ± 32.9/473 ± 22.8*</td>
<td>265 ± 33.0/350 ± 31.6</td>
<td>95.9 ± 15.0/112 ± 17.1</td>
</tr>
<tr>
<td>Total</td>
<td>1445 ± 51.2/1769 ± 38.2a*</td>
<td>902 ± 35.8a/1044 ± 62.0a*</td>
<td>543 ± 47.5/659 ± 42.9</td>
</tr>
<tr>
<td>10 g N m⁻²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First harvest</td>
<td>375 ± 20.2/486 ± 23.4a*</td>
<td>200 ± 12.5/296 ± 26.6*</td>
<td>175 ± 10.8b/190 ± 15.2</td>
</tr>
<tr>
<td>Second harvest</td>
<td>438 ± 13.2b/552 ± 22.3a*</td>
<td>263 ± 9.10b/337 ± 23.5*</td>
<td>175 ± 9.21/215 ± 17.4</td>
</tr>
<tr>
<td>Third harvest</td>
<td>473 ± 28.6b/548 ± 39.3a*</td>
<td>302 ± 20.1b/334 ± 24.2</td>
<td>171 ± 12.6/213 ± 26.6</td>
</tr>
<tr>
<td>Stubble</td>
<td>445 ± 40.0/422 ± 30.8</td>
<td>339 ± 32.5/301 ± 19.9</td>
<td>106 ± 12.8/121 ± 17.4</td>
</tr>
<tr>
<td>Total</td>
<td>1732 ± 49.0b/2008 ± 94.6b*</td>
<td>1112 ± 61.9b/1269 ± 78.8</td>
<td>627 ± 26.4/739 ± 66.0</td>
</tr>
</tbody>
</table>

Mean ± S.E. is shown. Total = stubble + three harvests. Values in a column followed by the asterisks are significantly different due to CO₂ treatment. Values in a column followed by the different letters are significantly different due to N treatment (two-way ANOVA).
level, respectively (Fig. 2). The N₂O fluxes were not significantly increased by the N fertilisation. On the first day after the first harvest followed by N fertilisation the N₂O was higher under ambient CO₂ at low and moderate N treatments (466 and 404 μg N₂O m⁻² h⁻¹, respectively) than that at elevated CO₂ at the low and moderate N treatment (266 and 46 μg N₂O m⁻² h⁻¹, respectively, Fig. 2). The difference was not statistically significant though (Fig. 2). In contrast, after the water table had been raised, the elevated CO₂ concentration seemed to increase the N₂O fluxes immediately after the harvest combined with the extra fertilisation (Fig. 2a). The difference was not statistically significant though (Fig. 2). In contrast, after the water table had been raised, the elevated CO₂ concentration seemed to increase the N₂O fluxes immediately after the harvest combined with the extra fertilisation (Fig. 2a). The difference was not statistically significant though (Fig. 2). In contrast, after the water table had been raised, the elevated CO₂ concentration seemed to increase the N₂O fluxes immediately after the harvest combined with the extra fertilisation (Fig. 2a). The difference was not statistically significant though (Fig. 2).

4. Discussion

4.1. Aboveground biomass and N yield

The total aboveground (three harvests + stubble) and harvestable dry biomass (three harvests) of the mixed Phleum/Trifolium stand increased under the elevated CO₂ concentration. According to Ainsworth and Long (2004), legumes produce more biomass than C₃ grasses under elevated CO₂, and the study of Sæbø and Mortensen (1995) shows that T. pratense can increase its dry weight production by as much as 30% due to elevated CO₂. Hebeisen et al. (1997) found that, in a bi-species mixed grass, T. repens markedly increased the harvestable biomass under elevated CO₂, whilst the harvestable biomass production of Lolium perenne decreased. In our study, both species increased the harvestable dry biomass by almost the same amount (ca. 20–25%) in response to the elevated CO₂ concentration. This emphasises how species-specific is the response to an enhanced supply of atmospheric CO₂. Even different Trifolium species can have either a negative or positive yield production response due to elevated CO₂ under the same treatments (Leadley et al., 1999).
The amount of harvested N also increased along with the increasing dry matter yield of the mixed mesocosms (Table 2), although the N concentration in the above-ground dry biomass clearly decreased with the moderate N supply, but not with the low N (Fig. 1). Both species reacted in the same way. One reason for the higher amount of harvested N under the elevated CO2 is probably the ability of T. pratense to fix N2, which can improve the availability of soil N to plants. Zanetti et al. (1996) showed that the total N yield increased under elevated CO2 and, additionally, that the assimilated N originated from N2 fixation by T. repens and not from the soil. Further, Zanetti et al. (1997) found that the total N yield of L. perenne grown in a mixture with T. repens under elevated CO2 concentration was higher than that of the monoculture. This finding is in accordance with our results. In our previous studies (Kettunen et al., 2005, 2006), both the N concentration and the amount of harvested N (g N m$^{-2}$) in

Fig. 2. Efflux of N$_2$O during the measurement periods and on the first and third day after harvest and N fertilisation in two CO2 and N treatments. The measurement periods after the first period start with harvest and N fertilisation; the small arrows indicate the time of harvest and N fertilisation. The asterisk indicates a statistically significant difference between the CO2 treatments ($P<0.05$, Mann–Whitney test) in N$_2$O fluxes before the first harvest. st. indicate the start of the experiment, sow the time of sowing and fer the time of the first N fertilisation. Mean±SE is shown; $n=9$. (a) 5 g N m$^{-2}$, (b) 10 g N m$^{-2}$.
the dry biomass of a *P. pratense* monoculture decreased significantly under the elevated CO$_2$ concentration. In contrast, when grown together with *T. pratense* in the present study, the amount of harvested N in the dry biomass of *P. pratense* increased with the same amount of N fertiliser. It is, however, worth bearing in mind that we used a closed system, which means that all the N that was supplied to the soil via fertilisation and via N$_2$-fixing by *T. pratense*, was used by the plants and soil microbes or sequestered into the soil or lost as N$_2$O or N$_2$, and no leaching occurred.

### 4.2. N$_2$O fluxes

The N$_2$O fluxes were highest in the beginning and at the end part of the experiment. In the beginning, immediately after germination of the seeds, N uptake by the plants was minor and hence more N was available for microbial use. However, under elevated CO$_2$ the germinated plants grew faster and consumed more N. Both *T. pratense* and *P. pratense* produced significantly more dry biomass and the amount of harvested N was higher at the first harvest under elevated CO$_2$ than at ambient CO$_2$ (Table 2, Fig. 1). Consequently, this was probably reflected as a significantly higher N$_2$O flux under ambient CO$_2$. This phenomenon is the same as that observed in our previous studies (see Kettunen et al., 2005, 2006). In our earlier experiment the difference between the CO$_2$ treatments was not as clear as now, which leads to the conclusion that the presence of a legume in the grass mixture affected the N$_2$O flux from the sandy soil already in the early stage of growth.

Under elevated CO$_2$ the mixed *Phleum/Trifolium* mesocosms showed no increase in N$_2$O fluxes during the periods with a low soil moisture content, and in fact there was a reduction in the N$_2$O fluxes (Fig. 2). Even after harvest followed by N application, there was no high peak of N$_2$O emissions under elevated CO$_2$, in contrast to that found in our earlier studies (see Kettunen et al., 2005, 2006). The elevated CO$_2$-induced increase in soil moisture, as was observed in our earlier study (see Kettunen et al., 2006), was inhibited by keeping the water level equally low in both CO$_2$ treatments during the basic experiment. Thus N$_2$O was most likely produced via nitrification, which does not benefit from an enhanced supply of organic C into the soil (Baggs et al., 2003a). Instead, the rate of nitrification is known to be increased in connection with disturbance, like clearing, cultivation and fertilisation of agriculture soils (Schlesinger, 1997). This could help to explain the high N$_2$O fluxes after the establishment of the experiment.

In the last part of the experiment, the level of the groundwater table was raised in order to create anaerobic conditions in connection with harvest and extra N application to promote denitrification. In addition, the increased root production of *P. pratense* under elevated CO$_2$ concentration, which was observed in our earlier studies with *P. pratense* at similar sandy soil (see Kettunen et al., 2006, 2007), may have supplied more new C in the soil. The majority of this new C is most likely rapidly cycled (Hungate et al., 1997; Niklaus et al., 2001; Xie et al., 2005; Hill et al., 2007), thus favouring denitrification together with the enhanced N$_2$ fixation by the legumes (Zanetti et al., 1996). Consequently, the rate of N$_2$O efflux was higher under elevated CO$_2$ concentration on the first and the third day after the harvest with N application (Fig. 2). However, the difference between the CO$_2$ treatments was not statistically significant due to the high variation in the N$_2$O fluxes, which is typical of soil N$_2$O emissions (van den Pol-van Dasselaar et al., 1998). Our results support the conclusions of Baggs et al. (2003b) and Niklaus et al. (2006) that N$_2$-fixing plants in grass systems have an impact on N$_2$O emission from the soil, but that the effect depends on the soil water status, soil nitrogen availability and also the atmospheric CO$_2$ level. The N and C cycles are closely linked and consequently an altered C supply may affect N dynamics in the soil, which in turn can affect plant growth under elevated CO$_2$ (Schneider et al., 2004). The plants can, in turn, change the soil moisture status (Arnone and Bohlen, 1998; Volk et al., 2000), which also can further alter the C and N cycles (Baggs et al., 2003a).

### 5. Conclusions

The results indicate that the increased supply of atmospheric CO$_2$ may reduce N$_2$O emissions from mineral soil under a mixed *Phleum/Trifolium* stand unless there are favourable conditions for denitrification, i.e. a high groundwater table. The higher burst of N$_2$O under elevated CO$_2$ in the last part of the experiment suggests that plant-derived C became available in the soil of mesocosms during the experiment, thus enhancing N$_2$O production when the conditions were favourable for denitrification. The dry matter yield production of the mixed *Phleum/Trifolium* stand under elevated CO$_2$ even increased with the low N treatment to the same level as the yield production with the moderate N treatment under ambient CO$_2$. Thus, the mixed growth of *Phleum/Trifolium* is able to utilise the increased supply of atmospheric CO$_2$ for enhanced biomass production without a simultaneous increase in the N$_2$O fluxes. This greenhouse experiment shows that, in the near future, more studies are needed concerning legumes in a grass mixture in order to clarify the possibility of maintaining N$_2$O emissions at their current level, while still enhancing the yield production even under low N fertiliser additions.

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