N_2O emission from grass-clover swards is largely unaffected by recently fixed N_2

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Biological N_2 fixation in grass-legume swards provides a major N input to many organic farming systems, but knowledge is sparse regarding the amount of fixed N_2 lost from the grasslands as N_2O. Nitrifying and denitrifying bacteria are the main contributors to the N_2O production in soils. According to the current guidelines issued by The Intergovernmental Panel on Climate Change (IPCC), biological N_2 fixation in grass-legume swards should not be considered as a source of N_2O in the national greenhouse gas inventories (IPCC, 1997), partly because of uncertainties in quantifying the N_2 fixation in the grasslands (Mosier et al., 1998). Hence, the agricultural greenhouse gas release may presently be underestimated. As organic farming to a very large extent utilises grass-legume mixtures as N source, the contribution from organic farming systems in particular may be underestimated. For all other N inputs (*viz.* inorganic fertiliser, manure and biological N_2 fixation in other crops), it is assumed that 1.25 % of the total N supply is emitted as N_2O (IPCC, 1997). This standard emission factor relies on experiments with fertiliser and manure only (Bouwman, 1996), and could thus be considerably unrepresentative for biologically fixed N_2. Therefore, as part of the DINOG project, a $^{15}N_2$-tracer-experiment was initiated, on grass-clover to assess the contribution of recently fixed N_2 as a source of N_2O and the transfer of fixed N from clover to companion grass.

Materials and methods

A mixture of white clover (*Trifolium repens* L. cv. Klondike) and perennial ryegrass (*Lolium perenne* L. cv. Fanda) was sown in pots using topsoil from an organic crop rotation. The ^{15}N-labelling approach consisted of enriching the atmosphere in a growth cabinet with $^{15}N_2$ (0.4 atom% excess) to trace the biological N_2 fixation (Fig. 1). A minimum-volume closed-system growth cabinet was developed, which could host 12 pots of 15 cm × 15 cm size. In this cabinet, three 14-day incubations were conducted with grass-clover mixtures at 16, 26 and 36 weeks of age. The N_2 fixation during the incubation was established by relating the excess ^{15}N

content of the plant and soil fractions to the ^{15}N enrichment of the atmospheric N_2. After the $^{15}N_2$-labelling, the emission of $^{15}N_2O$ was measured using a static chamber method (Fig. 2).

Fixation of nitrogen

At 16 weeks after emergence, N_2 fixation measured in grass-clover shoots and roots as well as in soil constituted 342 mg N m^{-2} d^{-1} (Fig. 3). This is three times larger than daily means of N_2 fixation determined in harvested herbage in the field (Jørgensen et al., 1999; Vinther and Jensen, 2000), probably because of optimal growth conditions at this stage of the experiment. Furthermore, our study estimates total amounts of fixed N in all pools, in contrast to the field measurements. Following a severe aphid attack, the N_2 fixation had dropped dramatically when measured at 26 weeks after emergence. Transfer of fixed N from clover to grass shoots was observed at 26 and 36 weeks to be 0.7 mg N m^{-2} d^{-1}, which accounted for 2 % of the N accumulated in grass shoots during the labelling period. In comparison, long-term field studies using ^{15}N dilution technique have reported apparent transfer of fixed N from white clover to companion ryegrass in the range 0 to 80 % of the grass N content (Boller and Nosberger, 1987; Ledgard, 1991), with the percentage increasing according to time after labelling.

Emission of N_2O

Total N_2O emission was 91, 416 and 259 µg N_2O-N m^{-2} d^{-1} at 16, 26 and 36 weeks after emergence, respectively (Fig. 4). To some extent, leaf insect pest status of clover seemed to influence the N_2O emission, probably by increasing death and decay of clover tissues. Emission of N_2O-N derived from recently fixed N_2 was not detected 26 or 36 weeks after emergence. At 16 weeks, only 3 ± 0.5 ppm of the recently fixed N_2 was emitted as N_2O on a daily basis, which represented 2 % of the total N_2O emission. Hence, the long-term mineralisation of dead clover tissues is most likely a more important source of N_2O than recently fixed N. Biological N_2 fixation in grass-legume swards should not be neglected as a source of N_2O in the national greenhouse gas inventories, especially not when considering the large area of Europe covered by managed grasslands. However, even though a longer time scale is taken into account, we find it unlikely that the N_2O emission factor for biologically fixed N_2 in grass-clover swards would reach the standard emission factor of 1.25 % suggested by IPCC. The reason is that only a part of the fixed N is mineralised during the lifetime of the clover, and furthermore that the release of inorganic N into the soil occurs slowly following decomposition of clover residues.

Conclusions

Our results indicate that N fixed within the previous two weeks constitutes about 2 % of the labile N pool in the soil. The data support the general view that recently fixed N contributes little to the N transfer from white clover to companion grass. Moreover, only a tiny fraction of the fixed N was lost as N_2O over the course of a few weeks. Thus, the long-term mineralisation of dead clover tissues is probably more important than recently fixed N for the flow from N_2 fixation to N_2O emission.

References

Boller, B. C. and Nösberger, J. (1987) Symbiotically fixed nitrogen from field-grown white and red clover mixed with ryegrasses at low levels of ^{15}N-fertilization. Plant Soil 104, 219-226.

Bouwman, A. F. (1996) Direct emission of nitrous oxide from agricultural soils. Nutr. Cycl. Agroecosys. 46, 53-70.

IPCC (1997) Greenhouse Gas Inventory Reference Manual. *In* Revised 1996 IPCC Guidelines for National Greenhouse Gas Inventories. *Eds*. Houghton, J. T. et al. Vol. 3. UK Meteorological Office, Bracknell.

Jørgensen, F. V., Jensen, E. S. and Schjoerring, J. K. (1999) Dinitrogen fixation in white clover grown in pure stand and mixture with ryegrass estimated by the immobilized ^{15}N isotope dilution method. Plant Soil 208, 293-305.

Ledgard, S. F. (1991) Transfer of fixed nitrogen from white clover to associated grasses in swards grazed by dairy cows, estimated using ^{15}N methods. Plant Soil 131, 215-223.

Mosier, A. et al. (1998) Closing the global N_2O budget: nitrous oxide emissions through the agricultural nitrogen cycle - OECD/IPCC/IEA phase II development of IPCC guidelines for national greenhouse gas inventory methodology. Nutr. Cycl. Agroecosys. 52, 225-248.

Vinther, F. P. and Jensen, E. S. (2000) Estimating legume N_2 fixation in grass-clover mixtures of a grazed organic cropping system using two ^{15}N methods. Agr. Ecosyst. Environ. 78, 139-147.