



Post-harvest N₂O and CO₂ emissions related to plant residue incorporation of oilseed rape and barley straw depend on soil NO₃⁻ content

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ABSTRACT

The sustainable production of bioenergy from crops like oilseed rape, barley, and maize presents a significant option to mitigate climate change by reducing fossil CO₂ emissions. Greenhouse gas emissions (specifically N₂O) during the energy crop production need to be quantified precisely for reliable life cycle analysis of bioenergy cropping systems. Energy crops (specifically oilseed rape) have a very high N demand, which results in a higher N-fertilizer application and thus higher risk of N₂O emissions not only during the vegetation period but also after crop harvest due to i) incorporation of N rich plant residue to soil and/or ii) residual N. An incubation experiment was conducted under conditions favoring denitrification (80% water-holding capacity), to study the drivers of N₂O emissions specifically during the post-harvest period. Here we compared two different plant residues varying in C/N ratio (oilseed rape (RST) and barley straw (BST)) with or without N supply and measured CO₂ and N₂O emissions. Stable isotope labeling (¹⁵N) was used to quantify soil- and residue-born N₂O. Incorporation of both plant residues alone induced significant increases in CO₂ emissions compared to control soil without straw addition ($p < .05$). However, the increase in CO₂ emissions was less pronounced when straw was incorporated in conjunction with mineral N. There was a clear increase in cumulative N₂O emissions ($p < .05$) when soil amended with BST or RST (6- and 9-fold) was compared to control, however, the increase of cumulative N₂O emissions was drastic when mineral N was added (15- and 23-fold). No significant differences in N₂O emission were observed when comparing residue types ($p > .05$). Stable isotope labeling of barley straw clearly showed that the share of residue-born N₂O was very low (1.35 or 0.4%) in the overall N₂O fluxes in BST and BST + N.

The present study suggests that N fertilization in autumn should be avoided to minimize N₂O fluxes regardless of type of straw.

1. Introduction

Renewable energies have gained great attention in policy making and the EU Renewable Energies Directive has been released aiming to increase the share of renewable sources in the energy supply to 20% (EU RED 2009/28/EC, 2009). The latter aims to reduce the consumption of fossil fuels and the emission of climate relevant carbon dioxide (CO₂). However, there is great concern that in the course of producing energy crops, the formation and emission of other potent greenhouse gases such as nitrous oxide (N₂O) would negate climate benefits. In northwestern Europe, oilseed rape based crop rotations have moved into focus as this crop has reached a large share in biodiesel production. Additional crops in oilseed rape crop rotations are the cereals barley

and wheat. So far, greenhouse gas (GHG) emissions in oilseed rape cropping systems seem to be greater than in others, e.g. cereal rotations (Walter et al., 2014).

After harvest, straw incorporation is a common and important agricultural practice to improve soil fertility. It improves soil physical conditions like aggregate stability and water infiltration and chemical properties like pH as well as macro- and micro-nutrient availability (Blanco-Canqui and Lal, 2009). Added straw provides a source of organic carbon and energy, but also a small share of nitrogen for decomposing soil microorganisms, which are essential for C and N mineralization (Chen et al., 2014a) as well as for vital soil microbial communities. Several studies show that the incorporation of plant residues into the soil increases both, biomass and activity of soil

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microorganisms (Blanco-Canqui and Lal, 2009; Potthoff et al., 2005; Rousk and Bååth, 2007), thus, contributing to CO₂ and also N₂O emissions from soil (e.g. Begum et al., 2014; Velthof et al., 2002). The velocity of CO₂ and N₂O emissions after straw incorporation depends on the mass and type of straw. Important characteristics are C and N concentrations of straw as well as the ratio of both (Huang et al., 2004). A low C/N ratio leads to the mobilization of straw C and N and its release into the soil mineral N pool which functions as substrates for microbial processes leading to CO₂ and N₂O emissions. Oilseed rape straw is ascribed a higher N concentration and a lower C/N ratio than barley straw which may accelerate its breakdown and its release of straw-N to the soil mineral N pool by soil microorganisms (Walter et al., 2014).

With respect to post-harvest processes in soil, there is another important post-harvest agricultural practice besides the incorporation of straw. Often N fertilizers are applied to foster straw mineralization and to warrant sufficient available N for the following crop. However, N addition to soil does not only promote residue mineralization and CO₂ production, but it may also favor nitrification and denitrification, potentially contributing to enhanced nitrate (NO₃⁻) leaching and N₂O release from soil to atmosphere. This may pose economic and environmental risks (Crutzen, 1970; Kaiser and Brenninkmeijer, 2002; Prather and Hsu, 2010). Additionally, N₂O is one of the most potent greenhouse gases with a global warming potential being 265 times higher than that of CO₂ on a 100-year-basis (Myhre et al., 2013).

To prevent the release of large amounts of N₂O, it is crucial to distinguish the various chemical and biological pathways forming N₂O. The most dominant biological processes are nitrification, denitrification and also nitrifier denitrification (Wrage et al., 2001). The microbial formation of N₂O and N₂ is also enhanced by specific soil physiochemical properties. García-Marco et al. (2014) list various factors like high NO₃⁻ availability and a C source leading to favorable conditions for N₂O production by denitrification. The addition of a labile C source, e.g. straw or root exudates can increase microbial activities as well as greenhouse gas emissions. This effect is more pronounced if the added C-source has a low C/N ratio, as shown by Huang et al. 2004. Nevertheless, also the addition of straw with a high C/N ratio leads to increasing N₂O emissions, although they are lower than emissions from soils amended with low C/N straw (Huang et al. 2004).

A number of studies have highlighted the connection of organic amendments to agricultural soil and its importance on soil N cycling (e.g. Chen et al., 2013; Chen et al., 2014a; Knorr et al., 2005; Thangarajan et al., 2013). However, there is little information on the release of residue N in form of N₂O (Frimpong and Baggs, 2010; Gentile et al., 2008; Millar and Baggs, 2005). So far, there is evidence that the increase of released N₂O might not be directly triggered by N released from straw which entered the soil mineral N pool (Frimpong and Baggs, 2010; Millar and Baggs, 2005). In the course of agricultural management, plant residues are regularly incorporated into the soil after harvest where they influence nitrogen turnover processes.

In this context, we have following hypothesis:

- (i) the annulment of N limitation will be followed by high soil CO₂ emissions and high soil mineral N content,
- (ii) the low C/N ratio of the oilseed rape residue will lead to higher N₂O emissions compared to barley straw with high C/N, and
- (iii) fertilizer N will promote the release of residue N as N₂O because of mineral N fluctuations that accelerate decomposition and soil N cycling.

For examining above mentioned hypothesis, we conducted an automated continuous flow incubation trial and monitored gas fluxes. In our incubation study, we focused on effects of incorporation of oilseed rape straw and barley straw on CO₂ emissions, soil N dynamics and N₂O emissions. In addition, N fertilizer application was considered to unravel potential nitrogen limitation of straw amended soils affecting the

release of CO₂ and N₂O. Stable isotope labelling approach was used to study the share of straw-N emitted as N₂O. In addition, we quantified the genes encoding the subunit of the nitrous oxide reductase (*nosZ*), responsible for the reduction of N₂O to N₂.

2. Material and methods

2.1. Experimental incubation set-up

A soil incubation experiment was carried out in a fully automated continuous flow incubation system using 15 incubation vessels of 20 cm diameter and 22 cm height. Soil was repacked (5.8 kg FW, 4.8 kg DM) into each incubation vessel including control soils (non-treated soil) to a final soil density of 0.96 g soil DM cm⁻³. The upper 15 cm of the mineral soil (Luvisol, clay 25%, silt 65.5%, sand 9.5%, pH_(CaCl2) 6.6, C 1.02%, N 0.11%) had been collected in spring 2013 from an unfertilized farmer's field in Sattenhausen close to Goettingen (51.51° N, 10.13° E). It was carefully air dried to allow sieving with a 4 mm mesh sieve. Complete drying out was avoided to minimize mineralization after rewetting.

To simulate good agricultural practice, ¹⁵N labeled barley (*Hordeum vulgare*, Total C: 41.17, Total N: 0.73, C/N: 56.45) or oilseed rape (*Brassica napus*, Total C: 43.97, Total N: 0.94, C/N: 46.61) straw was mixed with the upper 10 cm of soil prior to the experiment at a rate of 1.5 g straw DM kg⁻¹ soil DM. The straw was cut with scissors to a length of 2 cm to avoid large straw particles.

Prior to the experiment, 0.325 g of N was applied to the soil surface in the form of calcium ammonium nitrate (CAN, solid commercial fertilizer, 100 kg N ha⁻¹ equiv. to 67.5 mg N kg⁻¹ soil) in the respective treatments following rewetting of soil to c. 80% water holding capacity (WHC) by carefully dripping distilled water on the soil surface including the control. WHC was determined by putting a soil column in a cylindrical tube with a water-permeable membrane fixed underneath. The tube with the soil column placed into a water bath to saturate the soil column with water. Subsequently, the tube with the soil column was removed from the water bath and left for 24 h so that excessive water could run off through the membrane by gravity. By weighing, water content left in the soil column can be calculated. The amount of water left after 24 h is equal to 100% WHC. To adjust to 80% WHC, the water contents of the fresh soil and that of 80% WHC were used for calculation. Moisture conditions normally vary from year to year. To simulate frequently occurring moist conditions in autumn and to favor denitrification, WHC has been set to 80%. N addition reflects a high soil N level as typical fertilizer rates range from 30 to 40 kg N ha⁻¹ to agricultural fields in autumn.

All incubation vessels were sealed airtight and continuously flushed with synthetic air at a flow rate of 15 to 20 ml min⁻¹ to ensure aerobic conditions. The experiment was carried out in a temperature controlled environment at 22 °C and lasted for 43 days. For additional soil sampling a parallel system was set up under the same conditions in the same laboratory. Soil sampling was done every other day and after day 10, the time between sampling of soil was increased. Soil samples for molecular analysis were collected at day 1, 7, 11 and 25 and stored at -80 °C until further use. Overall, there were five soil treatments including non-treated control soil (CK), barley straw incorporation only (BST), oilseed rape straw incorporation only (RST), barley straw + N (BST + N), or oilseed rape straw + N (RST + N), all carried out in three replications.

2.2. Soil analysis

For determination of soil mineral N content, 9 g of soil FW were sampled and immediately processed to minimize mineralization. Samples were extracted with a 0.0125 M CaCl₂ solution (1:5 w/v) for 45 min. on an overhead shaker (85 rpm). The extracts were filtered with 615 ¼ filter paper (Macherey – Nagel GmbH & Co. KG, Düren,

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