



Pea cultivar and wheat residues affect carbon/nitrogen dynamics in pea-triticale intercropping: A microcosms approach



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HIGHLIGHTS

- Wheat residues have a large effect on the magnitude of C pools in soil under legume monocrop or intercrops.
- The addition of wheat residues can reduce N₂O fluxes.
- The choice of cultivar plays a key role on minimizing emission from the intercropping systems.
- Microbial activity was higher in the residue added treatment in whatever cropped legume systems.

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ABSTRACT

The underlying mechanisms by which legume cultivars contribute to nitrous oxide (N₂O) generation are poorly understood. The aim of the present study was to explore the effects of two pea cultivars (Zero4 and Nitouche) intercropped with triticale, with or without wheat (*Triticum aestivum*) residues incorporation, on soil C and N dynamics, on bacterial community structure and their links with N₂O emissions. Monocrops and bare soil (no plant) treatments were used as an additional control in order to account for the level of mineralisation between treatments. Changes in total C and N contents and in some functionally-related soil pools (microbial biomass C and N, basal respiration, KCl-exchangeable ammonium and nitrate, potentially mineralisable N, DOC, ecophysiological indexes) were followed throughout a 97-day microcosm experiment carried out on a loamy arable soil. ARISA community fingerprinting of soil extracted DNA and GHG emissions were carried out at two key stages (pea flowering and harvest). The addition of residues to the soil resulted in only small changes to the total C and N pools the Nitouche monocrop, which was found to have the highest potentially mineralisable N (13.4 $\mu\text{g g}^{-1} 28 \text{ d}^{-1}$) of the treatments with added residue. The different pea cultivar selectively affected N₂O emissions, with highest emissions associated with the cultivar Nitouche in the absence of residues. The two intercropping treatments of triticale/pea were significantly different either with residues or without, especially the triticale/Zero4 which had the lowest values (356 g N₂O-N ha⁻¹). Similar patterns were also observed in below ground data. ARISA analysis showed that monocropped legumes and the Triticale-based treatment clearly grouped on separate clusters to the added residue treatment. We hypothesize that in pea-based intercrops variations in carbon supply from different cultivars may contribute to differences in N₂O emissions and thus influence the choice of suitable cultivars, to optimize nutrient cycling and sustainable crop management.

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1. Introduction

Legume cropping offers opportunities to reduce GHG emissions from agriculture through their ability to substitute inputs of mineral fertilisers with biologically fixed N (Rochette and Janzen, 2005).

However, legumes differ widely in their contribution to N₂O emissions and in some cases (particularly following residue incorporation) can still remain a significant source (Baggs et al., 2000; Bouwman et al., 2002). The cultivation of leguminous crops in agricultural systems can not only contribute to reducing the emission of nitrous oxide (N₂O) but also increases the release and the turnover of mineralisable N-containing compounds in soil (Rochette and Janzen, 2005; Jensen et al., 2010). Their ability to add external N to the plant-soil system is a distinct benefit on which crop production systems can rely on in order to

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maintain the soil N supply at a sustained productive level (Watson et al., 2011). The amount of biologically fixed N supplied by legumes varies greatly from tens to several hundred kilograms per ha per year and is strongly affected by the type and environmental conditions (nitrate availability, temperature, soil wetness, and the availability of other nutrients).

Although symbiotic Rhizobium is believed to be able to produce N₂O in root nodules there is a conflicting evidence regarding the magnitude of this process. In their early work, O'Hara and Daniel (1985) suggested that rhizobial microorganisms are directly involved in the production of N₂O by reduction of NO₃ occurring within the root nodules. However, it is likely that Rhizobium species are not directly involved in the N₂O production process, and that the root microflora also plays an important role. Okubo et al. (2009) have shown that the rhizosphere community structure is significantly influenced by plant species and cultivar. It is also likely that this community structure is influenced by environmental conditions. It has been shown that different nodulation phenotypes contain different bacterial and fungal profiles in the stems and roots (Ikeda et al., 2008). However, the extent to which these phenotypes are associated with different emissions is unclear. In the case of legumes, it has been suggested that N₂O emission is primarily associated with decomposition and turnover of root nodules (Inaba et al., 2009), which implies that differences in the community structure and activity of root surface microorganisms may be responsible.

Understanding the contribution of legumes to N₂O emissions in the wider environment is highly dependent on developing an improved understanding of the underlying microbiology of the system (Philippot et al., 2002). Many studies have been conducted involving legume based cropping systems especially placed in intercrops or the growing of two or more species together at one time, since, legume-based intercropping is able to provide several agro-ecological services: a more efficient use of soil resources for plant growth due to a reduced competition for soil N (Hauggaard-Nielsen et al., 2003; Knudsen et al., 2004; Hauggaard-Nielsen and Jensen, 2005), an increased water and nutrient use efficiency (Hauggaard-Nielsen et al., 2009a), a greater yield stability and higher N concentration in cereal grain (Hauggaard-Nielsen et al., 2006, 2009b), a better control of soil erosion (Inal et al., 2007), and an enhanced weed suppression and pest control (Liebman and Dyck, 1993; Corre-Hellou et al., 2011). Moreover, reduced N₂O emissions from soil (Pappa et al., 2011) were also shown in leguminous intercrops. One more justification for intercropping (especially pea-based) is the increased mineral N made available in the soil for the following crop (Pappa et al., 2011; Scalise et al., 2015). Finally, the legume cultivar has been shown to play an important role in the cumulative N₂O emissions of the agricultural systems, which also affects the product intensities (Pappa et al., 2011), which are all the emissions divided by all saleable outputs.

The aim of this study was to explore the mechanisms responsible for N₂O emissions from two legume species demonstrated by Pappa et al. (2011) by monitoring a number of soil chemical (pH; EC; C_{org}; Nt; NH₄⁺-N; NO₃⁻-N; DOC), biochemical (MBC; R_{bas}; C₀, potentially mineralisable C; MBC/C_{org}; qM, mineralisation coefficient; qCO₂; qCO₂/C_{org} ratio; MBN; PMN, potentially mineralisable N) variables together with the bacterial community structure by ARISA fingerprinting of soil extracted DNA, and GHG emissions (N₂O, CH₄, CO₂) in an arable soil as by a microcosms approach.

The present study tested the following three hypotheses: a) legume-based cropping systems and wheat residue incorporation can stimulate soil C and N cycling through the enhancement of the below-ground nutrient flow, b) GHG emissions from legume-based intercropping can be altered by soil addition of wheat residues and c) even when showing a similar yield potential, the cultivar of a same leguminous species can selectively influence the soil processes including the bacterial community structure conditioned by the legume intercrop.

2. Materials and methods

2.1. Soil type and plant material

The soil used in the microcosm experiment was a loam collected from the Ap horizon (0–30 cm) of an agricultural field cultivated under continuous winter wheat and located at the Bush Estate, Edinburgh, Scotland (55°52'17.46" N, 3°12'24.27" W). The main soil properties were: sand 42%, silt 34%, clay 24%; bulk density 1.2 ± 0.1 kg dm⁻³; pH_{H2O} 6.19 ± 0.04; total organic C (C_{org}) 34.27 ± 1.22 g kg⁻¹; total N (N_t) 2.52 ± 0.08 g kg⁻¹; C:N ratio 13.62 ± 0.20; NH₄⁺-N 3.75 ± 0.40 mg kg⁻¹; NO₃⁻-N 7.64 ± 0.50 mg kg⁻¹; Olsen P 18.2 ± 0.4 mg kg⁻¹; extractable K 202.0 ± 0.3 mg kg⁻¹; electric conductivity measured in a soil:water (1:2, w/v) mixture (EC_{1:2} at 25 °C) 0.10 ± 0.01 dS m⁻¹. Following the winter wheat (*Triticum aestivum*) harvest (September 2011), residual straw was chopped to 2–4 mm and stored before being used for soil amendment. The soil for filling the microcosms was collected before starting the experiment (3rd October 2011), coarse sieved at <4.7-mm particle size and brought to approximately 30% gravimetric water content. Seeds of two cultivars of spring pea (*Pisum sativum* L. cv. Nitouche and *Pisum sativum* L. cv. Zero4) were provided by PGRO (UK); seeds of triticale (*Triticum aestivum* L. × *Triticosecāle* Wittm.) were provided by APVOSEMMENTI s.p.a. (Pavia, I).

2.2. Experimental set-up

The microcosm study was carried out at Scotland's Rural College (SRUC), in Edinburgh, between October 2011 and February 2012. Microcosm units consisted of 2.12 L polyvinyl chloride (PVC) pipes (25 cm height, 10.4 cm internal diameter) that had been closed at the base with an air-tight seal using a sheet of Plexiglas®. A sampling point for the gas collection (a three-way tap) was placed at 23 cm depth from the surface of the microcosm. Microcosms were filled either with soil (no residue addition) (unamended) or with a soil plus chopped wheat straw (400:1, w/w) mixture (corresponding to a 6.3 t ha⁻¹ addition rate at a field scale) (wheat residue addition) (amended).

The amount of soil needed was calculated by taking into account the microcosm volume (1867.92 cm³), the soil bulk density and the gravimetric water content in order to reach a water-filled pore space (WFPS) equal to 28–32% that provides optimum conditions for biological activity in soil (FAO, 2001). WFPS was kept constant during the growing season by watering with a N-free artificial rainwater (Palmqvist and Dahlman, 2006) in order to maintain suitable conditions for plant growth and microbial processes without providing an external N addition.

Soon after filling (7th October 2011), each microcosm, four seeds were initially sown but only two plants, of the same species or one of each intercrop components, were kept after successful seed germination. For each level of soil amendment, the following six treatments compared different combinations of leguminous intercrops and the respective sole crop: i) Nitouche: monocrop of pea cv. Nitouche; ii) Zero4: monocrop of pea cv. Zero4; iii) Triticale: monocrop of Triticale; iv) Nitouche-Triticale: intercrop pea cv. Nitouche-Triticale; v) Zero4-Triticale: intercrop pea cv. Zero4-Triticale and vi) bare soil: unplanted microcosms were used as a control.

Since the scheduled samplings were destructive, the whole experiment was duplicated, giving a total of 72 microcosms: (6 treatments) × (2 levels of amendment) × (2 samplings) × (3 replicates). The microcosms were randomly arranged in a growth chamber and grown for a 97-day growing period under controlled climatic conditions, as shown in Table 1.

2.3. Soil sampling and analysis

Soil samples were collected at three sampling times: at the beginning (pre-sowing), at pea flowering (62 days after sowing (DAS)) and

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