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

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RESEACH ARTICLE

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

Antonella Scalise[1,*,¶], Valentini A. Pappa[2,3,¶], Antonio Gelsomino[1], Robert M. Rees[2]

[1] Mediterranean University of Reggio Calabria, Department of Agricultural Sciences, Feo di 10 Vito, I-89124 Reggio Calabria, Italy

[2] SRUC, West Mains Road, Edinburgh, EH9 3JG, United Kingdom

[3] Texas A&M University, Energy Institute, 302 Williams Administration Building, College Station, TX-77843, USA

822 *Corresponding author (s). Antonella Scalise

823 E-mail address(es): antonella_scalise@msn.com (AS)

824 ¶ These authors contributed equally to this work

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Abstract

The underlying mechanisms by which legume cultivars contribute to nitrous oxide (N_2O) 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 N_2O emissions. Monocrops and bare soil (no plant) treatments were used as additional control in order to account for the level of mineralisation among 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 mineralizable N, DOC, ecophysiological indexes) were followed across a 97-day microcosms experiment carried out on loamy arable soil. ARISA community fingerprinting of soil extracted DNA and GHG emissions were also monitored 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 μg g^{-1} $28d^{-1}$) of the treatments with added residue. The different pea cultivar selectively affected N_2O emissions, with highest emissions associated with the cultivar Nitouche in the absence

Keywords

bacterial community structure, C and N pools, N_2O emissions, pea-based intercropping, wheat residues

841 of residues. The two intercropping treatments of triticale/pea were significantly different either with
842 residues or without, especially the triticale/Zero 4 which had the lowest values (356 g N_2O-N ha^{-1}).
843 Similar patterns were also observed in below ground data. ARISA analysis showed that monocropped
844 legumes and the Triticale-based treatment clearly grouped on separate clusters to the added residue
845 treatment. We hypothesize that in pea-based intercrops variations in carbon supply from different
846 cultivars may contribute to differences in N_2O emissions and thus influence the choice of suitable
847 cultivars, to optimize nutrient cycling and sustainable crop management.

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850 residues

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 the their contribution to N_2O 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 (N_2O) 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 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 symbiontic Rhizobium is believed to be able to produce N_2O 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 N_2O by reduction of NO_3 occurring within the root nodules. However, it is likely that Rhizobium species are not directly involved in the N_2O production process, and that the root microflora also plays an important role. Okubo *et al*. (2009) have shown that the rhizosphere community structure is

871 significantly influenced by plant species and cultivar. It is also likely that this community structure is
872 influenced by environmental conditions. It has been shown that different nodulation phenotypes
873 contain different bacterial and fungal profiles in the stems and roots (Ikeda *et al.*, 2008). However,
874 the extent to which these phenotypes are associated with different emissions is unclear. In the case of
875 legumes, it has been suggested that N_2O emission is primarily associated with decomposition and
876 turnover of root nodules (Inaba *et al.*, 2009), which implies that differences in the community
877 structure and activity of root surface microorganisms may be responsible.

878 Understanding the contribution of legumes to N_2O emissions in the wider environment is highly
879 dependent on developing an improved understanding of the underlying microbiology of the system
880 (Philippot *et al.*, 2002). Many studies have been conducted involving legume based cropping systems
881 especially placed in intercrops or the growing of two or more species together at one time, since,
882 legume-based intercropping is able to provide several agro-ecological services: a more efficient use
883 of soil resources for plant growth due to a reduced competition for soil N (Hauggaard-Nielsen *et al.*,
884 2003; Knudsen *et al.*, 2004; Hauggaard-Nielsen and Jensen, 2005), an increased water and nutrient
885 use efficiency (Hauggaard-Nielsen *et al.*, 2009a), a greater yield stability and higher N concentration
886 in cereal grain (Hauggaard-Nielsen *et al.*, 2006, 2009b), a better control of soil erosion (Inal *et al.*,
887 2007), and an enhanced weed suppression and pest control (Liebman and Dyck, 1993; Corre-Hellou
888 *et al.*, 2011). Moreover, reduced N2O emissions from soil (Pappa *et al.*, 2011) were also shown in
889 leguminous intercrops. One more justification for intercropping (especially pea-based) is the
890 increased mineral N made available in the soil for the following crop (Pappa *et al*., 2011; Scalise *et*
891 *al.*, 2015). Finally, the legume cultivar has been shown to play an important role in the cumulative
892 N_2O emissions of the agricultural systems, which also affects the product intensities (Pappa *et al*.,
893 2011), which are all the emissions divided by all saleable outputs.
894 The aim of this study was to explore the mechanisms responsible for N_2O emissions from two
895 legume species demonstrated by Pappa *et al*. (2011) by monitoring a number of soil chemical (pH;
896 EC; C_{org}; Nt; NH_4^+-N; NO_3^--N; DOC), biochemical (MBC; R_{bas}; C_0, potentially mineralisable C;
897 MBC/C_{org}; *q*M, mineralisation coefficient; qCO_2; qCO_2/C_{org} ratio; MBN; PMN, potentially

898 mineralisable N) variables together with the bacterial community structure

899 by ARISA fingerprinting of soil extracted DNA, and GHGs emissions (N_2O, CH_4, CO_2) in an arable

900 soil as by a microcosms approach.

901 The present study tested the following three hypotheses: a) legume-based cropping systems and

902 wheat residue incorporation can stimulate soil C and N cycling through the enhancement of the

903 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 Easter Bush, Edinburgh, Scotland (55°52'17.46" N, 3°12'24.27" W). 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; NH_4^+ - N 3.75 ± 0.40 mg kg^{-1}; NO_3^- - 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. x *Triticosecàle* Wittm.) were provided by APSOVSEMENTI s.p.a. (Pavia, I).

2.2 Experimental set-up

924 The microcosm study was carried out at Scotland's Rural College (SRUC), in Edinburgh, between
925 October 2011 and February 2012. Microcosm units consisted of 2.12 L polyvinyl chloride (PVC)
926 pipes (25 cm height, 10.4 cm internal diameter) that had been closed at the base with an air-tight seal
927 using a sheet of Plexiglas®. A sampling point for the gas collection (a three-way tap) was placed at
928 23-cm-depth from the surface of the microcosm. Microcosms were filled either with soil (no residue

929 addition) (unamended) or with a soil plus chopped wheat straw (400:1, w/w) mixture (corresponding
930 to a 6.3 t ha^{-1} addition rate at a field scale) (wheat residue addition) (amended).

931 The amount of soil needed was calculated by taking into account the microcosm volume (1867.92
932 cm^3), the soil bulk density and the gravimetric water content in order to reach a water-filled pore
933 space (WFPS) equal to 28-32% that provides optimum conditions for biological activity in soil (FAO,
934 2001). WFPS was kept constant during the growing season by watering with a N-free artificial
935 rainwater (Palmqvist and Dahlman, 2006) in order to maintain suitable conditions for plant growth
936 and microbial processes without providing an external N addition.

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

944 Since the scheduled samplings were destructive, the whole experiment was duplicated, giving a
945 total of 72 microcosms: (6 treatments) x (2 levels of amendment) x (2 samplings) x (3 replicates).
946 The microcosms were randomly arranged in a growth chamber and grown for a 97-day growing
954 rhizospheric soil was used for the molecular analysis and the bulk soil was used for the chemical and

947 period under controlled climatic conditions, as shown in Table 1.

948 *2.3Soil sampling and analysis*

949 Soil samples were collected at three sampling times: at the beginning (pre-sowing), at pea
950 flowering (62 days after sowing (DAS)) and at the pods filling pea stage (97 DAS), when the
951 microcosms were destructively sampled for soil and plant collection. Each microcosm provided one
952 rhizosphere sample (two samplings) and one bulk soil sample (three samplings). The rhizospheric
953 soil sample was taken from the plant roots after the bulk of the soil had been removed. The

954 rhizospheric soil was used for the molecular analysis and the bulk soil was used for the chemical and

955 biochemical characterization.

956 Soil chemical properties were determined according to standards methods recommended by the
957 Soil Science Society of America (Sparks, 1996). Dissolved organic carbon (DOC) was extracted with
958 water (1:2 w/v, soil:water) after shaking (170 rpm, 30 min) at room temperature. The soil slurries
959 were then centrifuged (4300 rpm, 10°C, 10 min) and the recovered supernatant was filtered through
960 a 0.45 µm Whatman GF/F membrane. DOC in the clean extract was finally measured using an
961 automated elemental OC analyzer (Rosemount-Dohrmann DC-80) (Jones *et al.*, 2005) using a
962 perchlorate oxidation followed by detection of CO_2 by NIR spectroscopy. Inorganic-N (NO_3^--N and
963 NH_4^+-N) was extracted with 1 M KCl (1:5, w/v, soil:solution) after shaking (220 rpm, 60 min) at
964 24°C. After the extraction, the soil slurries were centrifuged (4300 rpm, 10 min) and the clean
965 supernatants recovered and stored at -20°C before analysis. Inorganic N was determined using a
966 continuous flow auto-analyser (SKALAR San^{++}, BV, NL).

967 Microbial biomass C (MBC) and N (MBN) were determined following a chloroform fumigation-
968 extraction (CFE) procedure according to Vance *et al.* (1987) and Brookes *et al.* (1985). MBC was
969 estimated using a conversion factor of K_{EC} = 0.45 (Joergensen, 1996) and MBN was estimated using
970 a conversion factor of K_{EN} = 0.54 (Joergensen and Mueller, 1996). Soil basal respiration was
971 estimated by measuring CO_2 emissions in sealed 1.5 L jars containing 20 g (dw equivalent) soil
979 (Table Curve 2D v 5.01 software, SYSTAT software Inc.). Potentially mineralisable N (PMN),
980 resulting from net mineralization of active soil organic N occurring during the 28-day incubation

samples and incubated in the dark at 24 °C. Gas samples were collected in pre-evacuated 22 ml vials and analysed by gas chromatography (Sparling, 1981). The cumulative CO_2-C evolved after a 28-day incubation period (gas sampling was carried out after 1, 4, 7, 14, 21 and 28 days) was assumed as R_{bas}. The potentially mineralisable C (C_0) was estimated by fitting the 28-day cumulative data to the first-order exponential function $C_t=C_0\ (1-e^{-kt})$ (Riffaldi *et al*., 1996). The best fitting of the equation to the values experimentally obtained and estimates of C_0 and k parameters for each curve of basal respiration were obtained by non-linear regression analysis using the Levenburg-Marquardt algorithm (Table Curve 2D v 5.01 software, SYSTAT software Inc.). Potentially mineralisable N (PMN), resulting from net mineralization of active soil organic N occurring during the 28-day incubation

period for R_{bas} determination, was estimated as the cumulative inorganic soil N after 28 days *minus* the inorganic soil N at 0 day (Drinkwater *et al*., 1996). The following soil eco-physiological indices were then calculated: the microbial quotient (MBC/C_{org}), the metabolic quotient (qCO_2), the mineralization coefficient ($qM=R_{bas}/C_{org}$) and the qCO_2/C_{org} ratio (Dilly *et al.,* 2001; Mocali *et al*., 2009).

DNA extraction from both rhizosphere and bulk soil were undertaken by ball milling samples to achieve physical lysis followed by a CTAB-buffer extraction method as described by Brierley *et al.* (2009). DNA extracts were purified from any humic acids by passing them through micro Bio-spin columns loaded with polyvinylpyrrolidone (PVP). DNA yield and quality were quantified by a spectrophotometer (ND-1000). Automated ribosomal intergenic spacer analysis (ARISA) was carried with an end-point PCR technique using the primer system 1406f (5'-TGYACACACCGCCCGT-3') and 23Sr (5'-GGGTTBCCCCATTCRG-3'). The PCR reaction mixture was prepared with GoTaq® Green Master Mix (Promega), 2 µl of template DNA (ca 20 ng), 0.5 µM of each primer, and sterile deionised water to a final volume of 25 µl. In the negative control, the tDNA was substituted with the same volume of nuclease-free water (Promega). PCR running conditions started with a single denaturation step of 94 °C for 3 min, to activate the HotStart enzyme, followed by 29 thermal cycles

BioNumerics® 7.0 software package (AppliedMaths, Sint-Martens-Latem, B) as a 2D gel image for further analysis.

2.4 Greenhouse gas monitoring

Emissions of N_2O, carbon dioxide (CO_2) and methane (CH_4) from the microcosm units were

consisting of a denaturation step at 94 °C for 45 s, an annealing step at 55 °C for 1 min, and an elongation step at 72 °C for 2 min, followed by a final primer extension at 72 °C for 7 min and cooling to 4 °C. Capillary electophoresis with peaks ranging from 50-bp to 1,050-bp was carried out using an DNA 7500 assays on the Agilent 2100 Bioanalyzer (Analysis Software 2100, Agilent Technologies, Böblingen, D) according to manufacturer instructions. Electropherograms were imported into BioNumerics® 7.0 software package (AppliedMaths, Sint-Martens-Latem, B) as a 2D gel image for further analysis.

2.4 Greenhouse gas monitoring

Emissions of N_2O, carbon dioxide (CO_2) and methane (CH_4) from the microcosm units were

1007 measured following three gas sampling strategies: soil surface emissions, deep layer emissions (23
1008 cm) and respiration from roots. Surface gas monitoring started 12 days after sowing and was repeated
1009 (twice a week) across the entire experimental period by using the closed chamber technique (Smith
1010 *et al.,* 1995). During the gas emission measurements, the microcosms were covered by a 26-cm-tall
1011 chamber for 40-60 minutes before collecting 40 ml gas samples in a portable pre-evacuated 22-ml-
1012 glass vial (Scott *et al.,* 1999). For baseline corrections two air samples from the growing chamber
1013 atmosphere were collected at each sampling time. Gas sampling from deep soil layers started 38 days
1014 after sowing (14th November 2011) to allow time for the roots to grow throughout the microcosm and
1015 was repeated twice a week for three weeks. Gaseous emissions from legume roots collected after the
1016 microcosm destructive sampling (see below) were measured as described by Inaba *et al.* (2009).
1017 Shortly after the harvest, unwashed legume roots were placed into a 320 ml air-tight glass jars; 0 and
1018 10 min after sealing, a 40-ml-gas sample was collected from the glass jar and immediately transferred
1019 in a pre-evacuated 22 ml glass vial. All gas samples were stored (maximum 1 day) in a controlled
1020 temperature room before any analysis. Amounts of N_2O, CO_2 and CH_4 of collected air samples were
1021 analyzed using an Agilent 6890 gas chromatograph equipped with a 1.8 m Propak-N column and an
1022 electron capture detector (for N_2O) and flame ionisation detector (for CH_4). Certified high purity gas
1029 At pea flowering (62 DAS) and pod filling (97 DAS), the microcosms were destructively sampled,
1030 plants were gently removed from the microcosm soil and separated into shoot and root fractions.
1031 Shoot fresh weight was immediately recorded, whereas the root system was initially used for
1032 measuring the N_2O emissions (legumes only). The above ground biomass results were used for the

1023 standards of known concentration were used for calibration. The conversion of peak areas to daily
1024 gaseous emissions was carried out in accordance with standard procedures (de Kleine *and* Harvey,
1025 2013). In addition, greenhouse gas emission intensities were expressed per unit of product (all
1026 emissions divided by all saleable outputs. Also the Global Warming Potential (GWP) of each gas was
1027 calculated using coefficients of 1 for CO_2, 25 for CH_4 and 298 for N_2O.

1028 *2.5 Plant sampling and analysis*

1029 At pea flowering (62 DAS) and pod filling (97 DAS), the microcosms were destructively sampled,
1030 plants were gently removed from the microcosm soil and separated into shoot and root fractions.
1031 Shoot fresh weight was immediately recorded, whereas the root system was initially used for
1032 measuring the N_2O emissions (legumes only). The above ground biomass results were used for the

emission intensities calcilations.

2.6 Statistics

Soil variables were firstly checked for deviations from normality (Shapiro Wilk's test) and homogeneity of within-group variances (Levene's test). The block effect in the experimental design was not significant ($P > 0.05$) and the data were subjected to the following statistical analyses. A three-way analysis of variance (ANOVA) (treatment (T) x amendment (A) x time (Ti)), indicated in Figs. 1, 2 and 4 and in Table 2 as *F*-values and corresponding *P*-values, was performed in order to highlight the main effect of sampling time, crops, level of amendment and their interactions on measured soil variables. Significant effects due to treatment (T), amendment (A), and their interaction presented in Tab. 4 were estimated by a two-way ANOVA. Multiple pairwise comparison of means were assessed by Tukey's HSD (Honestly Significant Difference) test at $P < 0.05$ level of significance. Chemical and biochemical data were also analysed by principal component analysis (PCA) with no rotation with data from three different stages (pre-sowing, flowering and harvest) (Table 3 and 4). Statistical analyses were run using the Systat 11.0 software (SYSTAT Software Inc., Erkrath, D). Graphs were drawn by using the SigmaPlot 10.0 software (SYSTAT Software Inc.). Dendrograms of hierarchical classification of ARISA profiles were generated by cluster analysis using the unweighed pair-group method with arithmetic averages (UPGMA) based on Dice similarity coefficient as suggested by Rademaker *et al.* (1999).

soils, C_{org} values remained close to the initial values; whereas following wheat residue addition, a

3. Results

3.1 Soil C pools

Soil carbon pools showed variable responses to the addition of plant residues and the presence of different crop cultivar during the experiment. The addition of wheat residues in microcosm soils caused some significant reductions in the amount of the total organic carbon (C_{org}) (Table 2), although residue incorporation affected the C_{org} differently in treatments over time. In particular, in unamended

soils, C_{org} values remained close to the initial values; whereas following wheat residue addition, a

1058 contrasting affect was observed in C_{org} content between monocropped treatments were found to have

1059 the highest C_{org} concentrations. In bare soil C_{org} slightly declined, whereas it remained practically

1060 unaffected in amended ones.

1061 Dissolved organic carbon (DOC) varied in response to residue addition levels and sampling stages

1062 (Fig. 1). The presence of the intercrops increased the concentrations of DOC at harvest. Without

1063 residues addition, no significant difference was observed between treatments at any sampling stage;

1064 whereas following wheat residue addition the Zero4 treatment showed a significant increase ($P <$

1065 0.001) from pre-sowing (36.9 µg g^{-1}) to harvest (64.1 µg g^{-1}). On average, DOC increased over time

1066 from an initial value of 33.2 (or 37.3) to 48.2 (or 50.5) µg g^{-1} in unamended (or amended) microcosms

1067 soil, including the bare soil which showed an increasing trend over time.

1068 In unamended microcosms, mean soil basal respiration, R_{bas}, values were higher than pre-sowing

1069 at both flowering and harvest stage (respectively 778.9 and harvest 807.4 µg CO_2-C g^{-1} 28 d^{-1}) and

1070 there was no significant effect due to the crop treatment (Fig. 1). However, residue amendment

1071 strongly influenced ($P < 0.05$) the CO_2 emission of treatments at the harvest stage, which ranged

1072 between 553.5 (bare soil) and 1042.6 µg CO_2-C g^{-1} 28 d^{-1} (Nitouche monocropping): the Nitouche

1073 solo crop showed higher basal respiration than those at beginning of the experiment (from 721.7 to

1074 1042.6 µg CO_2-C g^{-1} 28 d^{-1}), whereas in the bare soil R_{bas} decreased by approximately 20% (from

1075 664.6 to 553.5 µg CO_2-C g^{-1} 28 d^{-1}). Estimates of the potentially mineralisable carbon (C_0) followed

the same general trend as those of R_{bas}, even though some of the experimental factors lost their significance (Fig. 1). It is noteworthy that C_0 displayed a time-dependent fluctuation with particularly high C mineralization from Triticale (differing from R_{bas}) and Nitouche monocrops.

Microbial biomass carbon (MBC) was strongly affected by treatments with statistically significant responses to all the experimental factors (Fig. 1). In general the MBC increased during the cropping season, in spite of residue amendment: from an initial 79.0 (or 75.5) to final 191.4 (or 243.6) μg C g^{-1} in unamended (or amended) soil microcosms. In soils with no wheat residue addition, MBC showed a large increase in the presence of legume-based treatments either in monocropped - from mean initial

79.1 to final 209.3 µg C g^{-1} (approximately +265%) - or intercropped legumes - from initial 75.5 to final 233.8 µg C g^{-1} (approximately +310%). An opposite affect was observed in residue amended soils: the MBC increase was generally lower under intercropping (+290%) than in monocropping (+390%) as compared with the starting value of 75.7 µg C g^{-1}.

3.2 Soil N pools

Time and time x amendment were the only factors that significantly affected the variability of total nitrogen content (N_t) in microcosms soils (Table 2). In fact, N_t content decreased across the 97-day experimental period with differing trends, but reaching similar values at the harvest stage (2.09 and 2.01 g kg^{-1} for unamended and amended, respectively) (data not shown). Across the experimental period, the extractable NH_4^+-N did not differ significantly in any of the treatments (Fig. 2); however, the amount of soil nitrate showed marked time-dependent fluctuations and was significantly different among treatments ($P < 0.001$). Crop growth markedly affected the dynamics of soil nitrate-N, which became greatly depleted at the flowering stage in all planted microcosms. An increased release of nitrate was observed at the latest stage, also mirrored by a decline in the ammonium-N content, yet regulated by the decaying wheat residues (Fig. 2).

The potentially mineralisable nitrogen (PMN) was affected by all the experimental factors and their interactions (Fig. 2). In general, PMN demonstrated a clear decrease from pre-sowing onward. At the flowering stage, PMN in the bare soil treatment was significantly higher ($P < 0.01$) than the harvest stage (12.3 µg g^{-1} 28 d^{-1}).

1102 treatments with no residue addition. It was noteworthy that, at the harvest stage, PMN was
1103 significantly affected by residue amendment, even though at a different level ($P < 0.05$ and $P < 0.001$,
1104 respectively). Specifically, Nitouche monocropping increased the PMN by three times from the
1105 flowering stage reaching the highest value of 13.4 $\mu g\ g^{-1}\ 28d^{-1}$ in microcosms packed without addition
1106 of wheat residues. All the remaining cropping treatments showed a small non-significant increase,
1107 but the bare soil retained similar values (10.81 $\mu g\ g^{-1}\ 28\ d^{-1}$). Further, in amended soils, there was a
1108 significant increase in the Triticale - Zero4 intercropping from the flowering (5.8 $\mu g\ g^{-1}\ 28\ d^{-1}$) to the

1109 harvest stage (12.3 $\mu g\ g^{-1}\ 28\ d^{-1}$).

All experimental factors and their interactions statistically influenced the microbial biomass N (P < 0.001). In unamended microcosms, MBN moderately (intercrops) or strongly (monocrops) increased over time, with the exception of the bare soil treatment where it decreased from the beginning of the experimental period (14.6 µg N g^{-1}) by approx. 40% (from 14.6 to 8.8 µg N g^{-1}). In contrast, in residue amended soils, the unplanted soil showed MBN values statistically comparable to other cropping treatments: as a whole MBN increased by approx. 70%, from initial 17.0 to final 28.8 µg N g^{-1} (Fig. 2).

3.3 Soil ecophysiological indices and C-to-N ratios

The mineralization coefficient (qM) was statistically influenced by the amendment level (P < 0.001), time (P < 0.001) and their interactions (Fig. 3). In unamended soils, the mineralization coefficient values showed a slight increase, on average from 16.79 µg CO_2–C mg^{-1} C_{org} (pre-sowing stage) to 24.35 µg CO_2–C mg^{-1} C_{org} (harvest sampling). In microcosms added with wheat residues, it showed an opposite trend for Zero4, Triticale and bare soil, which showed the major decline (from 25.14 µg CO_2–C mg^{-1} C_{org} to 18.80 µg CO_2–C mg^{-1} C_{org}).

The metabolic quotient ($q$$CO_2$) was significantly ($P$ < 0.001) affected only by time, level of soil amendment and their interaction (Fig. 3). Microcosms at both level of amendment showed a decrease in the values of the $q$$CO_2$ towards the end of the experiment, which was stronger in the amended soil due to the higher average values it showed in the pre-sowing stage (1.11 and 2.57 µg CO_2–C $µg^{-1}$ MBC d^{-1} respectively for unamended and amended). The largest decrease was registered in the

1129 Nitouche pure culture (from 2.69 to 0.19 μg CO_2–C μg^{-1} MBC d^{-1}). The qCO_2/C_{org} ratio was also

1130 statistically influenced by time ($P < 0.001$), level of soil amendment ($P < 0.001$) and their interactions

1131 (Fig. 3). However, in amended microcosms, the qCO_2/C_{org} ratio clearly decreased in all treatments

1132 from pre-sowing to harvest stage.

1133 The microbial quotient (MBC/C_{org}) was strongly ($P < 0.001$) affected by all the experimental

1134 factors (Fig. 3). MBC/C_{org} varied consistently during the experimental period and showed a marked

1135 increase at the harvest stage in all treatments at both amendment levels. The bare soil treatment always

showed the lowest value within each sampling time, reaching its minimum at the flowering stage in residue amended microcosms (2.09 µg MBC mg^{-1} C_{org}).

3.4 Soil pH and electrical conductivity

The three-way ANOVA revealed that wheat residue addition was the main factor affecting the variability of pH data ($P < 0.001$), which were generally higher in the amended soil (Table 2). There were also a time-dependent fluctuations ($P < 0.01$) together with significant effects of the amendment x time, and amendment x time x treatment interactions ($P < 0.001$). However, the pH varied between a narrow range comprised between 6.12 (unamended bare soil at flowering) and 6.37 (amended triticale at flowering), and significant differences among treatments were only noticed at the flowering and the harvest stages in the unamended soil with the Nitouche monocrop and bare soil having, respectively, the highest (6.39) and the lowest value (6.10).

The electrical conductivity ($EC_{1:2}$) varied between 0.10 and 0.18 $dS \cdot m^{-1}$ and was significantly affected by most of the experimental factors and their interactions (Table 2). It was noticeable that the triticale-based treatments showed higher $EC_{1:2}$ values than the leguminous sole treatments at both flowering and harvest stages: this finding was only observed in unamended, but not in the amended microcosms, and this was especially true for all crop-based treatments where EC remained almost constant over time. In the bare soil, the lowest EC was found in unamended treatments (~0.10 dS m^{-1}); whereas following wheat residues addition it increased considerably at flowering and harvest stage, respectively to 0.18 and 0.15 dS m^{-1}.

3.5 Multivariate analysis

1156 According to the eigenvalue > 1.0 criterion only five principal components could be selected. The
1157 first two principal components PC1 (eigenvalue 5.37) and PC2 (eigenvalue 2.79) explained a large
1158 portion (33.55 and 17.44%, respectively) of the total variance. The following three components PC3
1159 (eigenvalue 2.17), PC4 (eigenvalue 1.35) and PC5 (eigenvalue 1.06) accounted for 13.54, 8.43 and
1160 6.63% of total variance, respectively. Since the first two components taken together explained as

much as 50.98% of the total variance, we focused on them (Table 3). Firstly, it is worth noting that PC1 was primarily weighed by either C-related functional variables (DOC, *q*M, R_{bas}, MBC and MBC/C_{org}) or N-related variables (PMN and N_t). It was also found that PC2 was primarily affected by one of the most dynamic N pools in soil: NH_4^+-N, which was also directly related to qCO_2 and qCO_2/C_{org}. On the other hand MBN was the only variable affecting PC3. Moreover, PC4 was weighed by C_{org} and pH. Whereas, in PC5 PMC was the only soil variable showing a loading factor close to the reference threshold value (0.60). In the ordination biplot of Factor 1 vs Factor 2, soil samples from the differing treatments appeared in most cases well separated at least in three main groups along the PC axis 1 (functional C variables and N-related properties): triticale monocropping, Nitouche - Triticale intercropping and, surprisingly, a rather broad group including all the other crop treatments plus the bare soil. On the other hand, the two leguminous monocrops were clearly separated along the PC axis 2 (Fig. 4A).

The two first principal components PC1 (eigenvalue 5.21) and PC2 (eigenvalue 2.92) expressed a somewhat large portion (32.59 and 18.22%, respectively) of the total variance. The following three components PC3 (eigenvalue 2.04), PC4 (eigenvalue 1.46) and PC5 (eigenvalue 1.22) accounted for 12.77, 9.14 and 7.60% of total variance, respectively. Once again, we focused on the first two PCs as they explained as much as half of the variance (50.81%) (Table 4). PC1 was primarily weighed by

3.6 ARISA analysis

either C-related functional variables (MBC, MBC/C_{org}, qCO_2/C_{org}, qCO_2 and DOC) or N-related variables (MBN, PMN and N_t). PC2 was primarily affected by some C-related functional variables (R_{bas}, qM and C_0) and NO_3^--N. C_{org} was the only variable affecting PC3. EC was the only variable affecting PC4.. In the ordination biplot of Factor 1 vs Factor 2, soil samples from the amended microcosms were rather scattered onto the plot: the two intercropping combinations were closely associated, whereas the two leguminous monocrops were not. The bare soil was well separated from the other treatments. Noticeably, among the chemical and biochemical soil variables, NO_3^--N and C (C_0) exerted a primary role in separating the treatments along the PC2 axis (Fig. 4B).

3.6 ARISA analysis

1187 The molecular structure of the bacterial communities profiles were characterized by the number
1188 and length distribution of major bands which, in spite of treatments and residue levels, were observed
1189 in a fragment size range from 200 to 1000 bp, and showed a clear diversity between levels of residue.
1190 In particular, regardless of the growth stage, residues addiction in soils appeared to enhance the
1191 difference in groups allowing the monocropped legumes and Triticale-based treatment to clearly
1192 group on separate clusters (~78%; Fig. 5). On the contrary, in the no-residue soils, the treatment-
1193 dependent communities did not clearly align on the endemic axis, not allowing the clusters to present
1194 a clear pattern. The only clear difference was between bare soil and other treatments, which showed
1195 a level of similarity of approximately 73%.

1196 *3.7 Greenhouse gases (GHGs) emissions*

1197 N2O emissions from the amended treatments were lower in comparison to the unamended soils (P
1198 < 0.001). In the amended treatments, the emissions started to pick up after 60 days of the start of the
1199 experiment with the unplanted treatment having the highest emissions (81.25 g N_2O-N ha^{-1} day^{-1}). In
1200 the unamended treatments, the emissions were higher (P < 0.05) in the Triticale monocrop and
1201 Triticale/Nitouche treatments including also the no plant treatment from 30 days after the seeding.
1207 very low, and even showed consumption of N_2O (negative values) with similar patterns in the
1208 unamended soils (Triticale/Nitouche: 243 g N_2O-N ha^{-1} and Triticale/Zero4: -550 g N_2O-N ha^{-1})
1209 (Table 5). Below ground N_2O emissions showed a similar pattern between amendment levels during
1210 the experimental period. However, the concentration of N_2O was ten times greater from the no residue
1211 treatment in comparison with the residue (P < 0.001). The bare soil treatment had the highest average
1212 values (19.70 ppm and 1.95 ppm for the no residue and residue, respectively) followed by the

The cumulative values of N_2O were higher in the unamended treatments at 82 days. The bare soil treatment had the highest emissions in both treatments (4319 and 1430 g N_2O-N ha^{-1} in unamended and amended, respectively). In the microcosms with crop, the Triticale/Nitouche treatment had the highest (3677 g N_2O-N ha^{-1}) and the Triticale/Zero 4 the lowest (356 g N_2O-N ha^{-1}) emissions in unamended soils ($P < 0.001$). In the amended treatments, the cumulative emissions were generally very low, and even showed consumption of N_2O (negative values) with similar patterns in the unamended soils (Triticale/Nitouche: 243 g N_2O-N ha^{-1} and Triticale/Zero4: -550 g N_2O-N ha^{-1}) (Table 5). Below ground N_2O emissions showed a similar pattern between amendment levels during the experimental period. However, the concentration of N_2O was ten times greater from the no residue treatment in comparison with the residue ($P < 0.001$). The bare soil treatment had the highest average values (19.70 ppm and 1.95 ppm for the no residue and residue, respectively) followed by the

1213 Triticale/Nitouche treatment (2.56 and 1.30 ppm for the unamended and amended, respectively)

1214 (Table 5).

1215 Cumulative CO_2 emissions were highest in the Zero 4 treatment (2511 kg CO_2-C ha^{-1}) in unamended

1216 soils and the Nitouche (2790 kg CO_2-C ha^{-1}) under residue addition (Table 5). The bare soil treatment

1217 had the highest average belowground concentration of CO_2 in both residue treatments during the

1218 experimental period ($P < 0.001$) (Table 5). Methane emissions were low during the experimental

1219 period for both level of amendment without (Table 5).

1220 Emission intensities presented in this paper include the cumulative N_2O measurements (84 out of 97

1221 days) for the total biomass produced within this time providing an index of the effectiveness of

1222 mitigation. In the residue treatment, the Triticale/Zero4 had the lowest emission intensities of all the

1223 treatments (-393 g N_2O t $biomass^{-1}$). N_2O intensities were not significant different for the no residue

1224 treatment (Table 6).

1225 **4. Discussion**

1226 The results obtained from this study provide a new understanding of the interrelated effects of

1227 leguminous crops on the chemical and biochemical properties of soil and highlights the important

1234 Soil incorporation of wheat residues slightly reduced the total organic carbon (C_{org}), which

1235 appeared noticeable in the ANOVA analysis but resulted negligible impacts in the principal

1236 component analysis either with or without residue addition. Indeed, it could be anticipated that total

1237 soil organic matter, would not respond rapidly to environmental changes, unless major amendments

differences in C and N cycling associated with pea-based intercropping and wheat residue incorporation.

4.1 Soil chemical properties

Even in simplified ecosystems such as microcosms, soil organic carbon can be considered one of the most important indicators of soil quality because of its important role in the maintenance of soil structure, microorganisms and nutrient cycling (Aalders *et al*., 2009).

Soil incorporation of wheat residues slightly reduced the total organic carbon (C_{org}), which appeared noticeable in the ANOVA analysis but resulted negligible impacts in the principal component analysis either with or without residue addition. Indeed, it could be anticipated that total soil organic matter, would not respond rapidly to environmental changes, unless major amendments

1238 are made (Powlson *et al.,* 1987). However, mixing occurring during the establishment of the
1239 experimental units was expected to alter soil C dynamic and enhance rates of soil organic matter
1240 degradation, thus leading to a so-called tillage effect (Linsler *et al.,* 2013; Tortorella and Gelsomino,
1241 2011). This increased degradation activity not only influenced the carbon but also the nitrogen
1242 cycling, which is functionally interconnected in soil, and thus resulted in a more striking variation in
1243 N_t than in the C_{org}.

1244 Even if major changes in total organic carbon content may be difficult to detect over a short-term
1245 experiment (Haynes, 1999), the responses of more labile fractions of soil organic carbon, namely
1246 dissolved organic carbon (DOC), are much more sensitive to soil management than total soil organic
1247 matter (Silveira, 2005). This fraction markedly influences soil chemical, biological and physical
1248 properties, as a primary source of mineralizable C, N, P, and S (Haynes, 2000) and it has been
1249 proposed as an indicator of the size of the available C pool to soil microorganisms (Boyer and
1250 Groffman, 1996).

1251 Through their exudates, plant root systems represents a major source of C flow entering the soil
1252 and stimulating the microbial process of immobilisation/release of soluble organic compounds
1253 forming the DOC pool in soil (Paterson, 2003; Paterson *et al.,* 2007). In fact, the quality and amount
1254 of rhizodeposition released from the legumes root systems could explain the high significance showed
1255 by the crop factor on the variability of this parameter in this study (Fustec *et al*., 2010).

The addition of plant residues and fresh organic compounds through rhizodepositions most often results in a net N immobilisation phase followed by a net re-mineralisation phase. In our study, lower amounts of inorganic N were observed in the treatment with wheat residue addition than in the corresponding unamended treatment. Wheat residue incorporation seems to have enhanced net N immobilization, although N mineralization was promoted in presence of the legume treatment at the end of the incubation period.

The significant difference between amendment levels and crop presence shown suggests a different effect of faunal activity on residues. This could be due to increased available N in soil, which

1264 is consequently is not limiting for soil microorganisms responsible for degrading the residues.
1265 However, Knapp *et al*. (1983) reported conflicting evidence where some studies found mixed results
1266 from the effect of N availability on residue decomposition.

1267 Soil pH was fairly resilient to changes during the microcosm experiment (as clearly shown by PCA
1268 analysis) and this was actually not unexpected since it is not a highly variable parameter, and is often
1269 resilient also to short term perturbations (Table 3 and 4).

1270 *4.2 Soil biochemical properties*

1271 This study confirms, as previously suggested (Ndiaye *et al.*, 2000), that biological and biochemical
1272 parameters are more sensitive and can provide earlier measurements of changes produced by different
1273 soil and crop management than physical and chemical indicators. Most authors have studied the
1274 quantity and the activity of soil microbial biomass as indicator of changes driven by the addition of
1275 organic residue or cropping systems (Kaiser and Heinemeyer, 1993; Ndiaye *et al.*, 2000).

1276 Biochemical properties played and important role in explaining the response to treatments within
1277 this study being mainly related to the first principal components maintained in both amended and
1278 unamended soils.

1279 Microbial biomass, is known to be one of the main drivers of nutrient cycling in soils, with
1280 microbial activity releasing essential nutrients to plants and microbial biomass is functionally and
1281 closely linked to the turnover of soil organic carbon (Jenkinson and Ladd, 1981). It is therefore of
1289 mineralised before being assimilated by the newly-formed biomass. However, it has been shown that

1282 significance that the soil microbial biomass showed a greater increase, in all legume based treatments
1283 in the unamended soil. This increase, observed at the last sampling, could have been due to higher
1284 growth of microbial biomass, induced by the legume crop (Dinesh *et al*., 2004). The statistically
1285 significant difference shown in the microbial biomass dynamics in response to the presence/absence
1286 of residues can depend on the decomposition rate of plant material and on the microbial
1287 immobilisation processes. In fact, the N assimilation requirements are determined by this carbon flow
1288 (Mary *et al*., 1996). It is often assumed that N coming from the residue and from recycled biomass is

1289 mineralised before being assimilated by the newly-formed biomass. However, it has been shown that

1290 the soil microflora can directly assimilate significant amounts of organic N compounds coming from
1291 plant residues or from decaying biomass.
1292 Furthermore, the introduction of the residue amendment increased soil basal respiration as
1293 measured by cumulative CO_2 emissions. Although R_{bas} was not responsive to the individual
1294 treatments, it was markedly influenced by the interactions they determined with the amendment. This
1295 finding can suggest that in this soil the metabolic activity was primarily influenced by compositional
1296 changes in soil organic matter due an enhanced residue decomposition of the organic compounds
1297 released from plants roots.
1298 *4.3 Analysis of soil microbial community structures*
1299 The results obtained in this study confirm that the addition to the soil of crop residues can strongly
1300 modify the genetic structure of the community by stimulating particular populations; especially as
1301 the soil system is often substrate-limited as regards microbial growth (Nicolardot *et al*., 2007). In fact,
1302 the molecular analysis revealed that the genetic structures of the bacterial population itself were
1303 significantly changed in response to the presence of legume sole crops or triticale, either in association
1304 with the legume or in monocropping, as a function of the presence/absence of wheat residue in the
1305 soils.
1306 *4.4 Gas emissions*
1307 It has been demonstrated that there were lower N_2O emissions from legumes, which is consistent
1308 with our understanding that there are low levels of N_2O emission associated with the fixation process
1309 (Rochette *and* Janzen 2005). Our study has repeated the observation of Pappa *et al* (2011) showing

higher emissions from the pea cultivar Nitouche both as a monocrop and when grown as an intercrop. Monocrop Nitouche (434 g ha^{-1}) had up to six time higher emissions than the monocrop Zero 4 (71 g ha^{-1}) in the amended treatment and twice in the unamended (749 and 374 g ha^{-1} for Nitouche and Zero 4, respectively). However, there was no significant difference between the intercropping treatments. Intriguingly these higher emissions were observed in the absence of wheat residue additions, and did

not appear to be associated with elevated concentrations of DOC. The denitrification processes driven by the availability of oxidisable carbon, which is used as a terminal electron acceptor in the respiratory process. Therefore, the absence of higher levels of DOC in the legumes was elevated emissions of N_2O raises the possibility that the carbon was being supplied by the legume itself. Support for this hypothesis would be provided by higher soil respiration rates measured from Nitouche, even in the absence of acid plant residues and as indicated in differences in microbial activity shown by the ARISA analysis.

Plant species and combinations of species offer significant opportunities to modify soil derived N_2O emissions. Selecting specific legume cultivars in combination with cereals may therefore provide a key to the mitigation of N_2O emissions. It is possible that the mechanisms underlying these differences would be associated either with an improved capacity of certain legume cultivars to compete more efficiently for soil N. Alternatively there may be an interaction between the legume and soil microbial community that reduces N_2O emission (possibly by promoting increased rates of N immobilization). The choice of legume cultivar and species is therefore a key factor influencing the amount of N loss. A previous study (Pappa *et al*., 2011) has shown that the cultivar Zero 4 has significant lower N loss by N_2O emissions and leaching and could therefore contribute to the development of agricultural systems with environmental benefits. Therefore having a better understanding of the varietal differences in selecting intercrops mixtures has a high potential to increase yields and contribute towards the developments of agricultural systems with environmental

benefits.

5. Conclusions

Legumes are generally associated with lower emissions of N_2O than cereal crops. However, there is significant variability in emissions between different legume cultivars. In this study the higher emissions associated with Nitouche were generated in the absence of wheat residues, raising the possibility that this variation in emissions is driven by variations in carbon supplied from the legume root. The intercrop affect on microbial activity is also cultivar specific. This is indicated by differences

1341 in N_2O emissions observed from two pea cultivars when grown as intercrops, although differences in
1342 N_2O emission were not linked to differences in yield. The mechanism underlying these differences
1343 appears to be driven by the differences resulting from microbial activity, which in turn are likely to
1344 be linked to soil-plant carbon dynamics.
1345 Our research therefore highlights the importance of the cultivar choice in the sustainable
1346 agricultural systems. The addition of the residues affects the soil C pools and the N_2O emissions and
1347 shows clear differences between the two pea cultivars but also the intercropping combinations. The
1348 root development of pea monocrops was influenced by the residue addition but also the presence of
1349 cereal highlighting the complexity of such systems. The scale of these effects is highly sensitive to
1350 management and soil type. The growing need for environmental tests of the legume cultivars to
1351 understand further the mechanisms of the GHGs emissions is in high priority. Long term further
1352 studies should be conducted to gain more information on the soil-plant-microbe system about the
1353 fates of C and N by adding 15N and 13C labelled residues of different species. Understanding the
1354 development of legume cultivar and the interactions taking place within legume/cereal intercrop has
1355 the potential to be a very useful management tool in the development of more sustainable agricultural

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systems and in mitigation of GHG from agriculture.

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1507 of ley duration on the yield and quality of the subsequent cereal crop (spring oats) in an organically
1508 managed long-term crop rotation experiment. Org. Agric. 1, 147-159.
1509

1510 **Table 1** – Chamber growth conditions during the 97-day experimental period were in accordance to

1511 the 26-year average climatic data recorded between April and August in a Mediterranean environment

1512 (Reggio Calabria, Southern Italy). The relative humidity was kept stable at 70%. Lighting was

1513 produced by cool white fluorescent bulbs at an average intensity of 1160 lux.

Growth period (day)	Temperature (°C)		Photoperiod (h) Day/Night
	Day	Night	
0-20	18.2 ± 0.3	10.2 ± 0.3	6.5/17.5
21-40	23.4 ± 0.3	14.6 ± 0.2	8/16
41-60	28.0 ± 0.3	18.9 ± 0.3	9.5/14.5
61-80	30.6 ± 0.3	21.7 ± 0.2	10.5/13.5
81-97	31.2 ± 0.4	22.5 ± 0.2	9.5/14.5

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Table 2 – Soil pH, EC, organic C and total N in the soil (mean ± SD, *n = 3*) was measured at the beginning and at the end of the experimental period. Symbols – and + represent absence or presence of amendment in soils. For each sampling time, different letters in the columns indicate significant differences among treatments (Tukey's HSD test at $P < 0.05$). Significant effects due to treatment, amendment, time and their interactions on the variability of data (*F*-value from three-way ANOVA, treatment x amendment x time, with corresponding *P* values[a]) are also shown at the bottom.

	Treatment		pH	$EC_{1:2}$ (dS m^{-1})	C_{org} (mg g^{-1})	N_t (mg g^{-1})
Pre-sowing	Nitouche	-	6.19 ± 0.06	0.10 ± 0.01	34.13 ± 1.75	2.50 ± 0.10
		+	6.24 ± 0.07	0.11 ± 0.01	29.94 ± 5.34	2.33 ± 0.29
	Zero4	-	6.17 ± 0.04	0.11 ± 0.01	34.13 ± 1.75	2.50 ± 0.10
		+	6.29 ± 0.03	0.11 ± 0.01	28.97 ± 4.41	2.33 ± 0.28
	Triticale	-	6.21 ± 0.04	0.10 ± 0.01	34.87 ± 0.92	2.53 ± 0.06
		+	6.23 ± 0.06	0.11 ± 0.01	30.07 ± 5.48	2.50 ± 0.20
	Triticale/Nitouche	-	6.18 ± 0.04	0.10 ± 0.01	34.40 ± 0.26	2.53 ± 0.06
		+	6.26 ± 0.08	0.11 ± 0.01	33.73 ± 1.60	2.60 ± 0.10
	Triticale/Zero4	-	6.19 ± 0.06	0.10 ± 0.01	33.83 ± 1.24	2.50 ± 0.10
		+	6.24 ± 0.07	0.11 ± 0.01	33.47 ± 2.06	2.60 ± 0.11
	Bare soil	-	6.21 ± 0.04	0.11 ± 0.01	34.27 ± 1.76	2.53 ± 0.12
		+	6.28 ± 0.03	0.11 ± 0.01	29.97 ± 5.58	2.50 ± 0.10
Harvest	Nitouche	-	6.31 ± 0.04 [a]	0.11 ± 0.01 [b,c]	36.36 ± 4.56	2.15 ± 0.02
		+	6.25 ± 0.03	0.12 ± 0.01 [a,b]	35.03 ± 7.51	2.12 ± 0.15
	Zero4	-	6.24 ± 0.04 [a,b]	0.10 ± 0.01 [c]	32.19 ± 0.92	2.06 ± 0.03
		+	6.27 ± 0.04	0.12 ± 0.02 [b]	37.54 ± 3.51	2.07 ± 0.01
	Triticale	-	6.18 ± 0.03 [a,b]	0.13 ± 0.01 [a]	30.32 ± 1.37	2.08 ± 0.06
		+	6.31 ± 0.03	0.10 ± 0.01 [b]	33.62 ± 2.31	2.05 ± 0.06
	Triticale/Nitouche	-	6.21 ± 0.03 [a,b]	0.12 ± 0.01 [a,b]	36.96 ± 1.52	2.09 ± 0.10
		+	6.30 ± 0.03	0.11 ± 0.01 [b]	28.12 ± 2.12	1.87 ± 0.09
	Triticale/Zero4	-	6.21 ± 0.02 [a,b]	0.12 ± 0.01 [a,b]	34.62 ± 1.06	2.10 ± 0.01
		+	6.28 ± 0.07	0.10 ± 0.01 [b]	30.58 ± 2.05	1.96 ± 0.19
	Bare soil	-	6.16 ± 0.01 [b]	0.10 ± 0.01 [c]	30.98 ± 0.43	2.09 ± 0.07
		+	6.29 ± 0.05	0.15 ± 0.01 [a]	28.89 ± 1.30	1.98 ± 0.32
	Factor	**df**				
	Treatment (T)	5	1.866 [ns]	1.866 [ns]	1.172 [ns]	0.510 [ns]
	Amendment (A)	1	67.903 [***]	67.903 [***]	5.815 [*]	2.276 [ns]
	Time (Ti)	2	5.026 [**]	5.026 [**]	2.238 [ns]	121.046 [***]
	T x A	5	8.130 [***]	8.130 [***]	0.558 [ns]	0.146 [ns]
	T x Ti	10	1.350 [ns]	1.350 [ns]	1.571 [ns]	1.204 [ns]
	Ti x A	2	0.500 [ns]	0.500 [ns]	1.982 [ns]	16.213 [***]
	T x A x Ti	10	3.024 [**]	3.024 [**]	2.897 [**]	1.051 [ns]
	Error	72				

[a] Levels of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns: not significant.

Table 3 – Soil Principal component analysis (PCA) of 16 soil chemical and biochemical variables measured in the six experimental treatments (Nitouche, Zero4, Triticale, Nitouche - Triticale, Zero4 - Triticale, Bare soil as in Materials and Methods) in the unamended soils during the 97-day microcosm experiment. PC loading variables (values ≥ |0.60| are in bold) and percent of total variance explained by the first five factors (eigenvalue >1) are reported. Soil variables are as described in Materials and Methods.

Soil variable	PC1	PC2	PC3	PC4	PC5
PMN	**-0.82**	-0.32	-0.11	-0.06	-0.32
N_t	**-0.82**	-0.24	0.14	0.01	-0.13
R_{bas}	**0.82**	0.15	-0.39	-0.18	-0.23
MBC/C_{org}	**0.80**	-0.35	0.27	0.23	0.14
MBC	**0.79**	-0.34	0.34	0.11	0.14
***q*M**	**0.75**	0.07	-0.54	0.11	-0.15
DOC	**0.73**	0.34	0.28	0.07	0.11
C_0	0.58	-0.07	-0.34	-0.23	**-0.60**
NH_4^+-N	-0.01	**0.83**	0.06	-0.23	-0.19
***q*CO_2**	-0.24	**0.78**	-0.14	0.26	0.17
***q*CO_2/ C_{org}**	-0.20	**0.72**	-0.27	0.37	0.13
MBN	0.37	0.01	**0.70**	0.12	-0.17
NO_3^--N	0.40	-0.45	-0.50	0.10	0.25
C_{org}	-0.01	0.13	0.49	**-0.63**	0.03

pH	0.09	0.15	0.45	**0.60**	-0.52
EC	0.48	0.44	0.17	-0.37	0.12
Variance explained (%)	*33.55*	*17.44*	*13.54*	*8.43*	*6.63*

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Table 4 – Principal component analysis (PCA) of 16 soil chemical and biochemical variables measured in the six experimental treatments (Nitouche, Zero4, Triticale, Nitouche - Triticale, Zero4 - Triticale, Bare soil as in Materials and Methods) in the amended soils during the 97-day microcosm experiment. PC loading variables (values ≥ $\left|0.60\right|$ are in bold) and percent of total variance explained by the first five factors (eigenvalue >1) are reported. Soil variables are as described in Materials and Methods.

NH_4^+-N	-0.21	0.53	0.39	-0.20	-0.41
C_{org}	0.18	0.17	**0.68**	0.19	0.56
EC	-0.01	0.06	0.03	**0.91**	-0.28
pH	0.13	-0.07	0.48	-0.06	-0.02
Variance explained (%)	*32.59*	*18.22*	*12.77*	*9.14*	*7.60*

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Table 5 – Average above ground flux emissions (µg ml^{-1}) for the whole experimental period for N_2O, CO_2 and CH_4 followed by carbon dioxide equivalent, expressed in t, and then average below ground flux emissions (µg ml^{-1}) for the whole experimental period for N_2O, CO_2 and CH_4. Symbols – and + represent absence or presence of amendment in soils. Significant effects due to treatment, amendment and their interaction on the variability of soil data (*F*-values from two-way ANOVA, treatment x amendment, with corresponding *P* values[b]) are also shown at the bottom.

Treatment		Above ground			Below ground		
		N_2O	CO_2	CH_4	N_2O	CO_2	CH_4
Nitouche	-	0.39 ± 0.16[b]	1620.34 ± 1022.89	2.10 ± 0.81	0.47 ± 0.04[b]	2805.17 ± 639.15[b]	2.34 ± 0.13
	+	0.38 ± 0.10	2253.45 ± 1608.46	2.10 ± 0.78	0.59 ± 0.12[b]	5075.28 ± 701.74[b]	2.17 ± 0.11[b]
Zero4	-	0.33 ± 0.11[b]	2076.57 ± 1869.64	2.07 ± 0.81	0.66 ± 0.11[b]	4386.84 ± 719.72[b]	2.00 ± 0.14
	+	0.34 ± 0.09	2219.74 ± 1795.18	2.06 ± 0.86	0.72 ± 0.15[b]	7073.73 ± 992.48[b]	2.05 ± 0.14[b]
Triticale	-	0.59 ± 0.55[a,b]	2077.48 ± 1704.66	2.12 ± 0.51	2.08 ± 1.00[b]	3048.84 ± 447.91[b]	2.12 ± 0.15
	+	0.37 ± 0.15	2156.48 ± 1722.16	2.14 ± 0.86	0.56 ± 0.08[b]	10373.01 ± 1115.54[b]	2.26 ± 0.09[b]
Triticale/Nitouche	-	0.86 ± 0.38[a]	1920.04 ± 1616.19	2.35 ± 0.67	2.56 ± 1.17[b]	7799.00 ± 1167.89[b]	2.10 ± 0.14
	+	0.30 ± 0.11	2243.43 ± 1870.47	2.05 ± 0.86	1.30 ± 0.42[a,b]	7714.24 ± 1584.48[b]	2.29 ± 0.09[b]
Triticale/Zero4	-	0.38 ± 0.07[a,b]	2054.57 ± 1878.98	2.11 ± 0.78	0.87 ± 0.33[b]	3725.17 ± 415.45[b]	2.23 ± 0.12
	+	0.36 ± 0.10	2388.89 ± 2103.67	2.07 ± 0.82	0.67 ± 0.14[b]	7743.60 ± 683.45[b]	2.18 ± 0.12[b]
Bare soil	-	0.80 ± 0.33[a,b]	1687.66 ± 1205.27	2.17 ± 0.73	19.70 ± 4.76[a]	7907.47 ± 1193.37[a]	2.31 ± 0.16
	+	0.36 ± 0.08	2389.69 ± 2266.00	2.14 ± 0.89	1.95 ± 0.37[a]	17480.05 ± 1398.95[a]	3.28 ± 0.37[a]
Factor	**df**						
Treatment (T)	**5**	3.286 **	0.050 ns	0.062 ns	12.008 ***	14.550 ***	4.173 **
Amendment (A)	**1**	18.408 ***	1.064 ns	0.141 ns	13.033 **	44.209 ***	3.216 ns
T x A	**5**	4.116 **	0.083 ns	0.093 ns	9.221 ***	5.023 ***	2.374 *
Error	**84**						

[a] Different letters in a column indicate significant differences among treatments (Tukey's test at $P < 0.05$).
[b] Levels of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns: not significant.

Table 6 – Emission intensities (total cumulative N_2O measurements divided by the total biomass for the whole experimental period), expressed in g per t of total biomass. Symbols – and + represent absence or presence of amendment in soils. Significant effects due to treatment, amendment and their interaction on the variability of soil data (*F*-values from two-way ANOVA, treatment x amendment, with corresponding *P* values[b]) are also shown.

Treatment		Intensities		
		N_2O	CO_2	CH_4
Nitouche	-	0.04 ± 0.41	219.30 ± 302.67	171.22 ± 62.25
	+	0.01 ± 0.04	933.45 ± 258.87	143.11 ± 98.77
Zero4	-	0.92 ± 0.33	1066.66 ± 201.18	182.58 ± 153.10
	+	-0.02 ± 0.48	1119.81 ± 647.68	58.53 ± 175.01
Triticale	-	1.33 ± 0.84	898.48 ± 789.79	199.41 ± 310.07
	+	-0.11 ± 0.36	1159.32 ± 256.00	462.84 ± 691.76
Triticale/Nitouche	-	0.68 ± 1.15	774.83 ± 1465.21	166.11 ± 167.84
	+	0.24 ± 0.35	2104.40 ± 1861.03	543.02 ± 879.48
Triticale/Zero4	-	0.18 ± 0.40	846.15 ± 47.80	355.79 ± 44.58
	+	-0.30 ± 0.31	1492.26 ± 1035.41	180.94 ± 409.14
Factor	**df**			
Treatment (T)	**4**	1,754 ^{ns}	0,603 ^{ns}	0,566 ^{ns}
Amendment (A)	**1**	8,884 **	2,008 ^{ns}	0,098 ^{ns}
T x A	**4**	2,463 ^{ns}	0,219 ^{ns}	0,462 ^{ns}
Error	**18**			

[a] Different letters in a column indicate significant differences among treatments (Tukey's test at $P < 0.05$).

[b] Levels of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns: not significant.

Figure captions

Fig. 1. Changes in soil dissolved organic C (DOC), basal respiration (R_{bas}), potential mineralisable C (C_0) and microbial biomass C (MBC) (mean ± SD, *n=3*) in unamended (left) and amended (right) microcosm soils at three sampling times (0, 62 and 97 DAS) over the 97-day experimental period for the six treatments: Nitouche, Zero4, Triticale, Triticale-Nitouche, Triticale-Zero4, bare soil.

Fig. 2. Changes in KCl-extractable ammonium-N (NH_4^+-N), KCl-extractable nitrate-N (NO_3^--N), potential mineralisable N (PMN) and microbial biomass N (MBN) (mean ± SD, *n=3*) in unamended (left) and amended (right) microcosm soils at three sampling times (0, 62 and 97 DAS) over the 97-day experimental period. Treatments are as in Fig. 1.

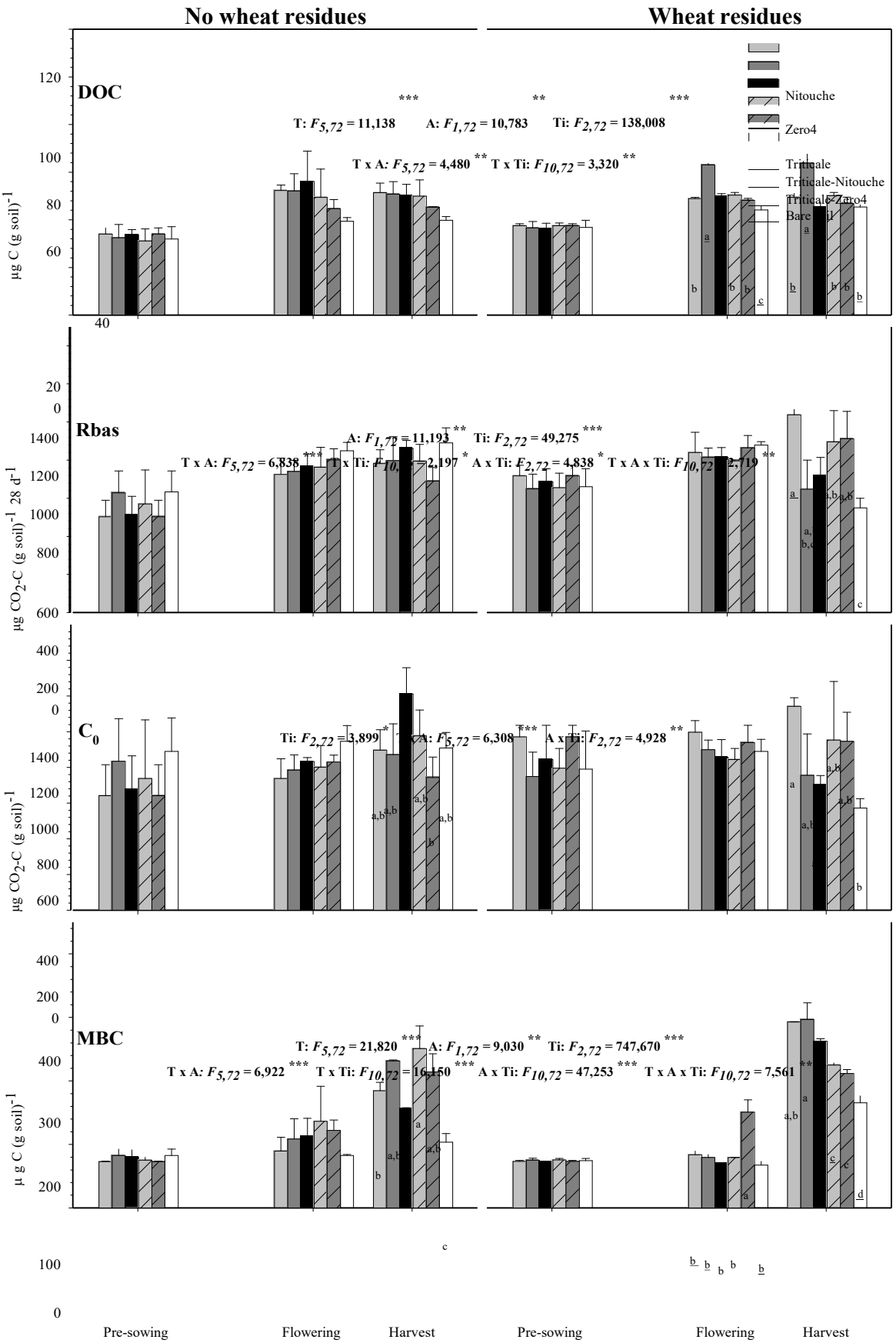
Fig. 3. Changes in mineralization coefficient (*q*M), metabolic quotient (qCO_2), qCO_2/C_{org} ratio and microbial quotient (MBC/C_{org}) (mean ± SD, *n=3*) in unamended (left) and amended (right) microcosm soils at three sampling times (0, 62 and 97 DAS) over the 97-day experimental period. Treatments are as in Fig. 1.

Fig. 4. PCA ordination biplot (PC1 *vs* PC2) of 16 soil chemical and biochemical variables (loadings, see Materials and Methods) measured in the six experimental treatments (Nitouche, Zero4, Triticale, Triticale-Nitouche, Triticale-Zero4, bare soil as in Materials and Methods) (scores) at three sampling times (pre-sowing, flowering, harvest) in the unamended (A) and the amended soils (B) during the 97-day microcosm experiment. The biplot has the same origin for

scores and loadings.

Fig. 5. Hierarchical classification (Pearson's similarity coefficient, Ward's clustering method) of banding patterns generated by ARISA of PCR-amplified 16S rRNA gene-coding fragments from soil-extracted bacterial DNA from no-residue (A) and residue (B) added microcosms at two sampling times (62 and 97 DAS) over the 97-day experimental period. Treatments are as in Fig. 1. Each bar averages three microcosm replicates. Scale bar (0–100) indicates the similarity level.

1580 **Figure 1 (C pools)**



Samplin
g stage

1583 **Figure 2 (N pools)**

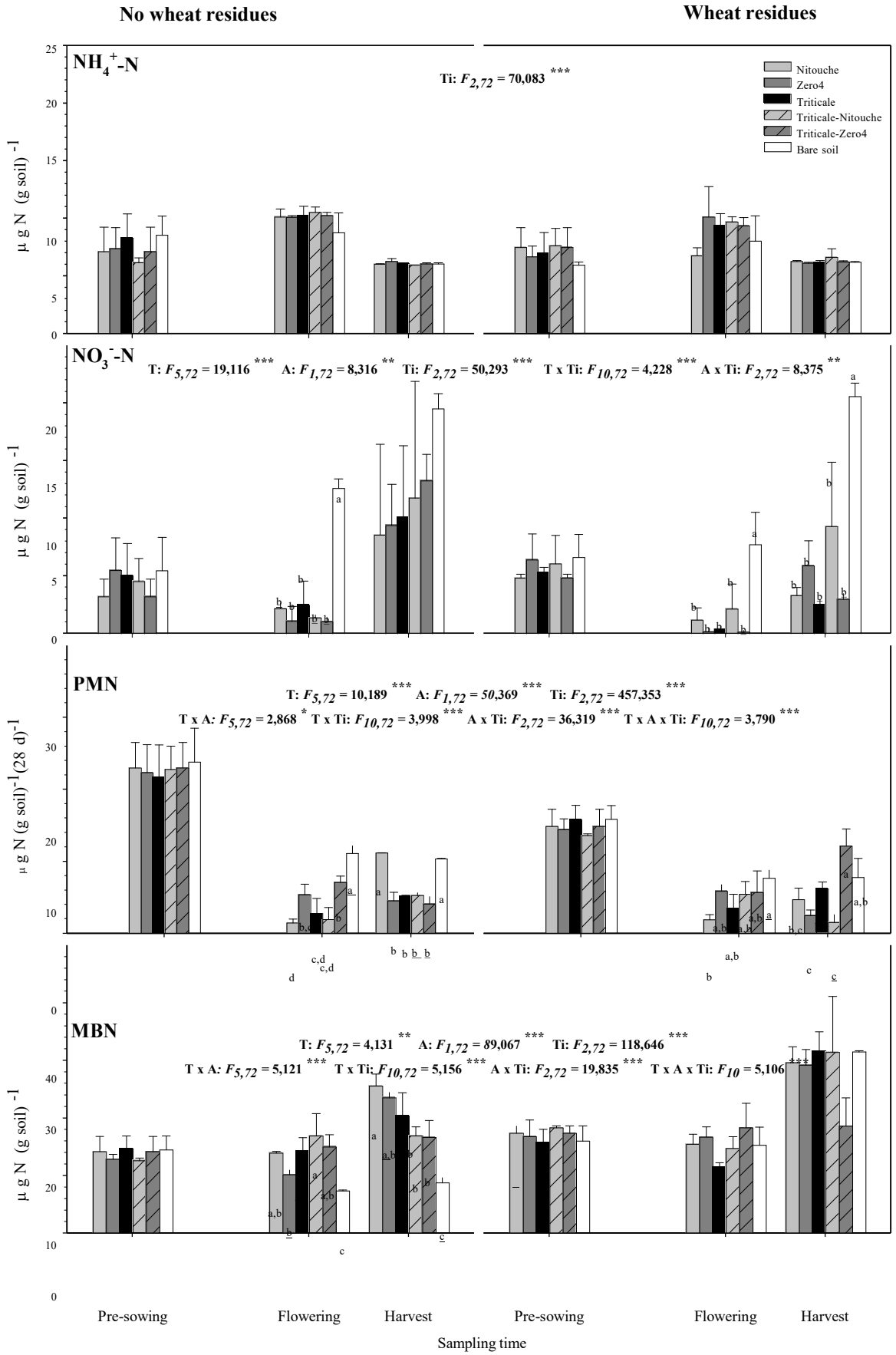


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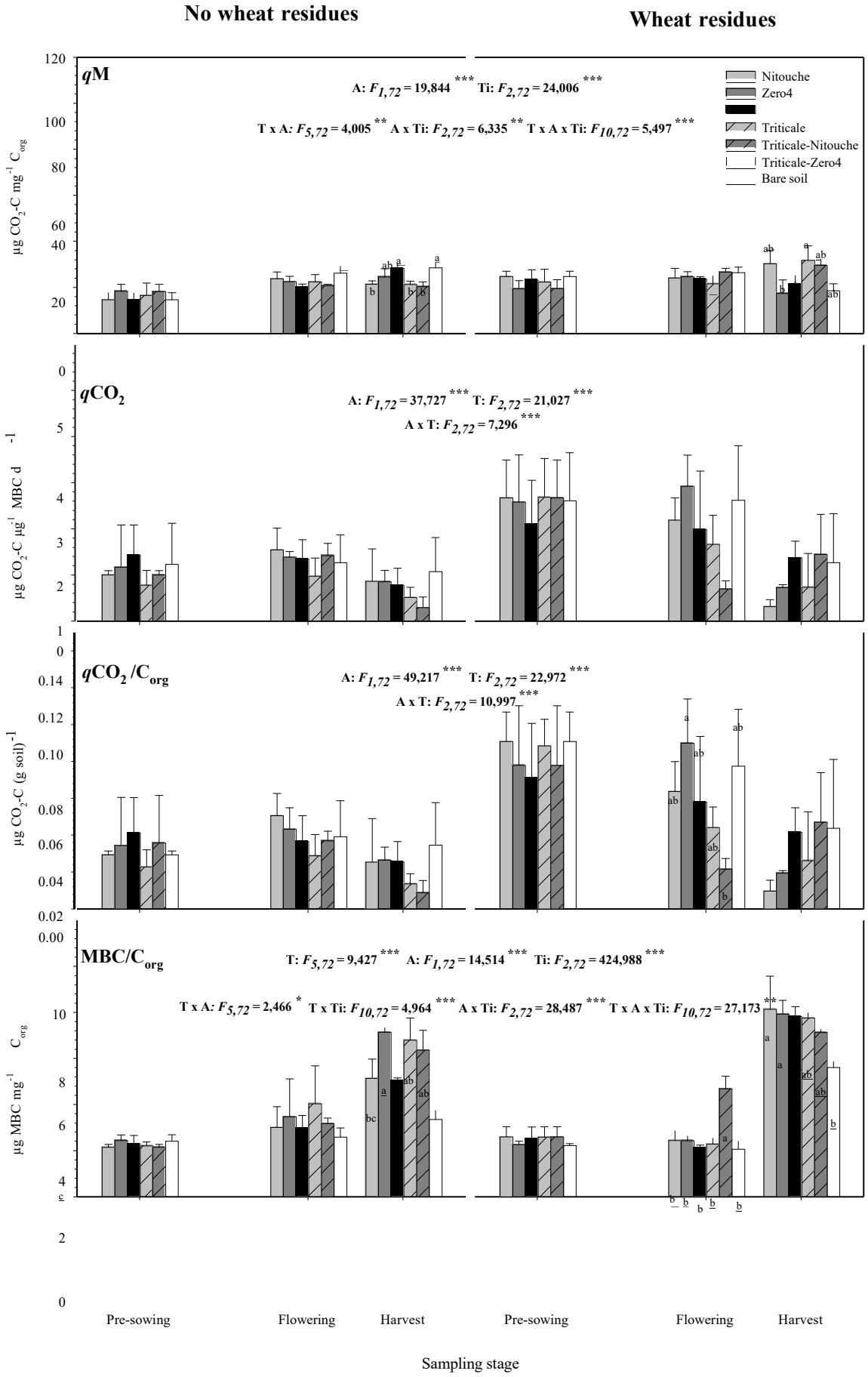
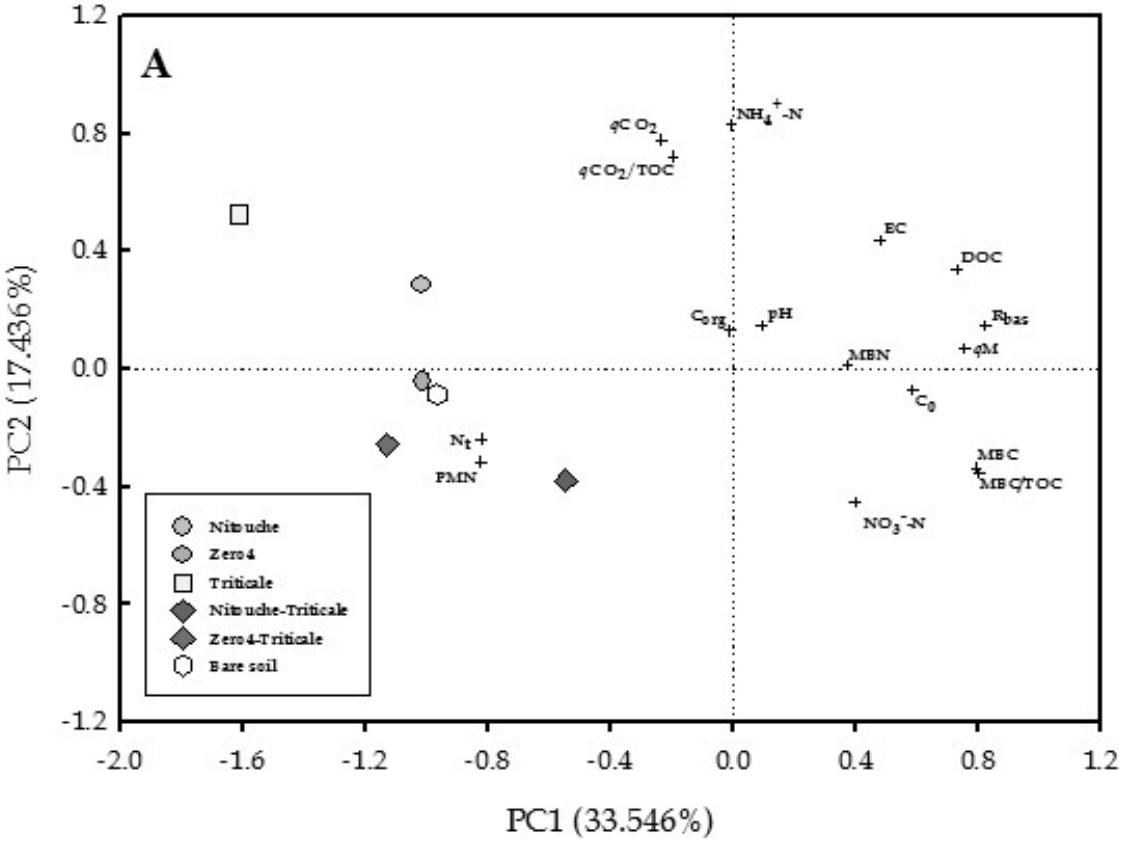
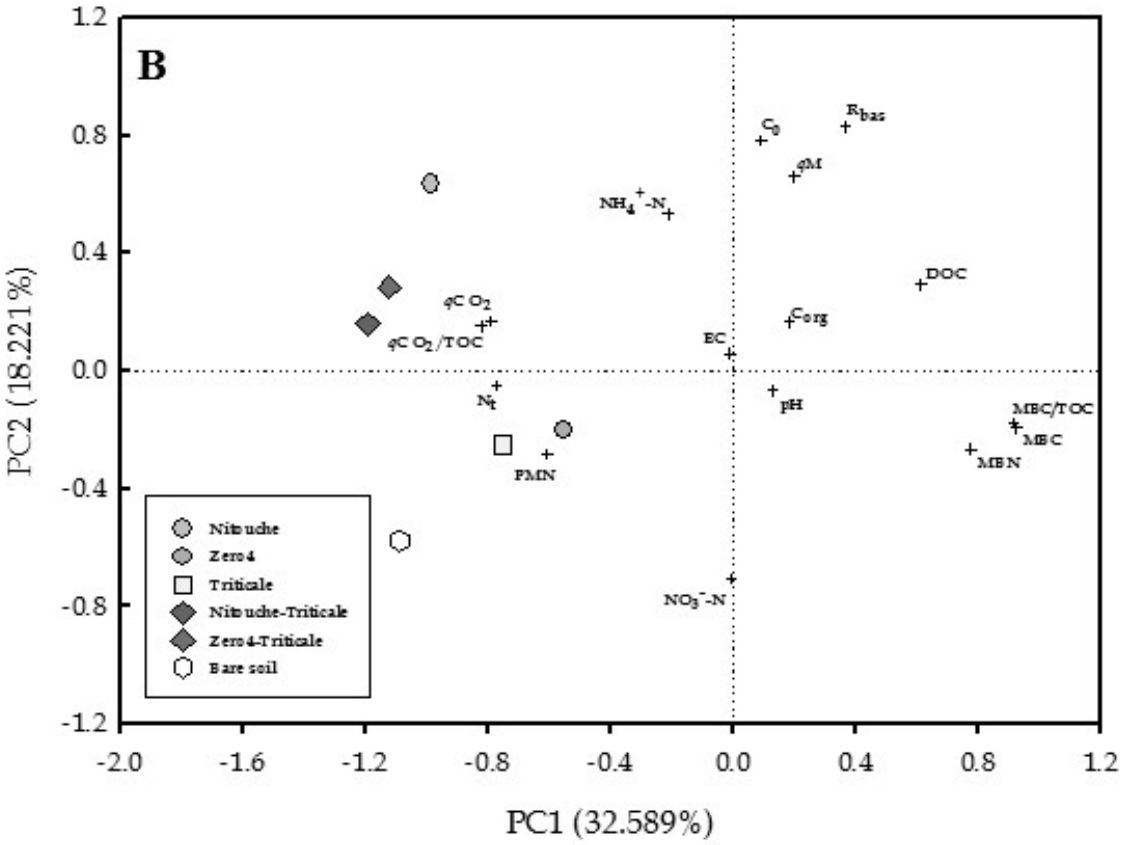
Figure 3 (Ecophysiological indices)



1589 **Figure 4 (PCA analysis)**





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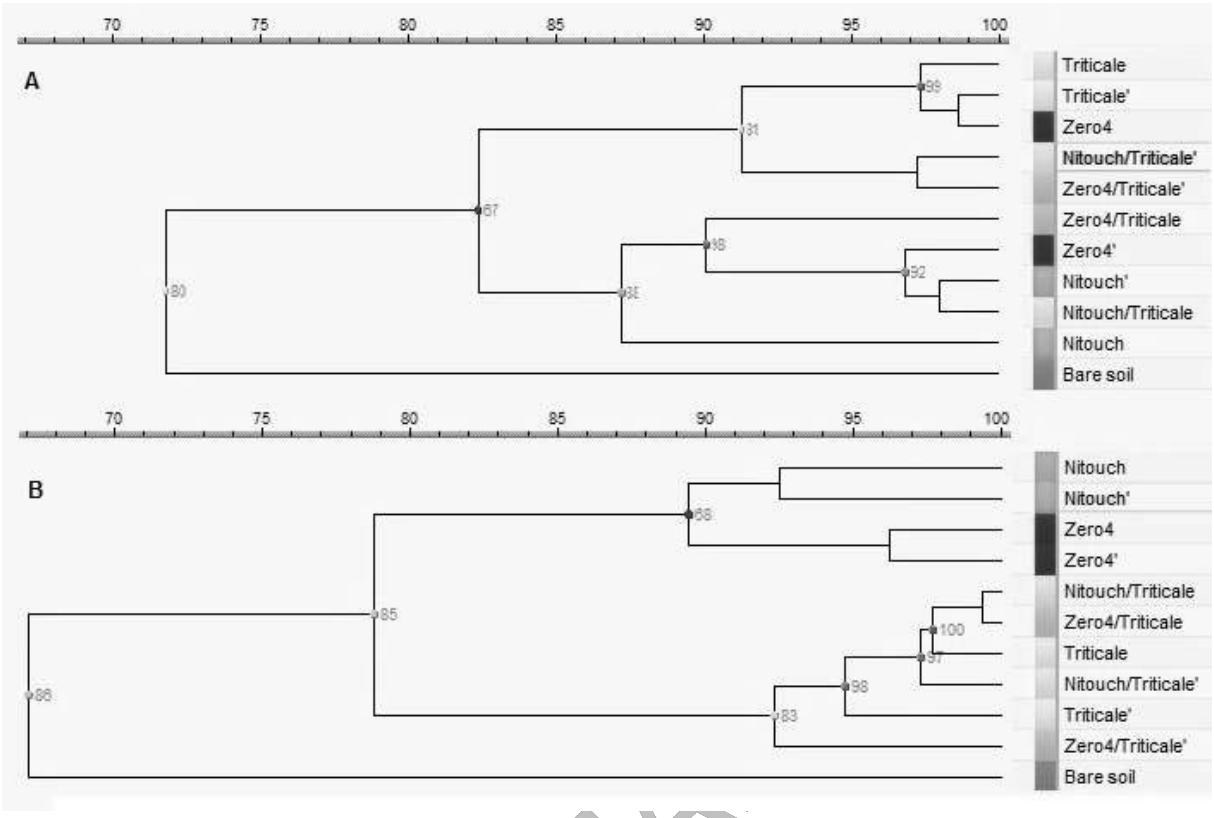
1592 **Figure 5 (ARISA analysis)**



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