https://doi.org/10.1016/j.agee.2014.03.023

Tillage and nitrogen fertilization effects on nitrous oxide yield-scaled emissions in a rainfed Mediterranean area

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Abstract

There is a strong need to identify the combination of tillage and N fertilization practices that reduce the amount of nitrous oxide (N_2O) emissions while maintaining crop productivity in dryland Mediterranean areas. We measured the fluxes of N_2O in two field experiments with 3 and 15 years since their establishment. In the long-term experiment, two types of tillage (NT, no-tillage, and CT, conventional intensive tillage) and three mineral N fertilization rates (0, 60 and 120 kg N ha^{-1}) were compared. In the short-term experiment, the same tillage systems (CT and NT) and three N fertilization doses (0, 75 and 150 kg N ha^{-1}) and two types of fertilizers (mineral N and organic N with pig slurry) were compared. N_2O emissions, water-filled pore space, soil mineral N content, grain yields, N-biomass inputs and soil total nitrogen (STN) stocks were quantified and the N_2O yield-scaled ratio as kg of CO_2 equivalents per kg of grain produced was calculated. In both experiments tillage treatments significantly affected the dynamics of N_2O fluxes. Cumulative losses of N as N_2O were similar between tillage treatments in the long-term field experiment. Contrarily, although not significant, cumulative N losses were about 35% greater under NT than CT in the short-term experiment. NT significantly increased the production of grain and the inputs of N to the soil as above-ground biomass in both experiments. Averaged across fertilizer treatments, CT emitted 0.362 and 0.104 kg CO_2 eq. kg $grain^{-1}$ in the long-term and the short-term experiment, respectively, significantly more than NT that emitted 0.033 and 0.056 kg CO_2 eq. kg $grain^{-1}$, respectively. Nitrogen fertilization rates did not affect the average N_2O fluxes or the total N losses during the period of gas measurement in the long-term experiment. Contrarily, in the short-term experiment, N_2O emissions increased with application rate for both mineral and organic fertilizers. The use of pig slurry increased grain production when compared with the mineral N treatment, thus reducing the yield-scaled emissions of N_2O by 44%. Our results showed that in rainfed Mediterranean agroecosystems, the use of NT and pig slurry are effective means of yield-scaled N_2O emissions reduction.

Keywords

Nitrous oxide; Mediterranean; nitrogen fertilization; tillage; soil organic nitrogen; yield-scaled N_2O emissions.

1. Introduction

Human activities impact the N cycle through the production and use of fertilizers and fossil fuel combustion (Galloway et al., 2004). Agricultural and natural N inputs to the biosphere from N fertilizers, animal manure, biological N_2 fixation and atmospheric N deposition increased from 155 to 345 Tg N yr^{-1} between 1900 and 2000 (Bouwman et al., 2013). That increase entails major losses of N from the agricultural systems such as nitrate leaching, erosion and gaseous emissions by denitrification. Among them, denitrification is the major terrestrial N removal process (Bouwman et al., 2013; Seitzinger et al., 2006).

The emission of N to the atmosphere as nitrous oxide (N_2O) has received recent attention due to its role as a powerful greenhouse gas (GHG) with a global warming potential (GWP) 298 times greater than the carbon dioxide (CO_2) (Forster et al., 2007) and its involvement in the depletion of the ozone (O_3) layer in the stratosphere that could result in harmful effects due to solar ultraviolet radiation (Crutzen, 1974). The transformation of N to N_2O has been related mainly to two biological processes, i.e. the loss of N as N_2O during the nitrification of NH_4^+ under aerobic conditions, and the reduction of NO_3^- under anaerobic conditions. Together, these processes account for 70% of global N_2O emissions (Braker and Conrad, 2011). Other processes such as chemodenitrification, chemical decomposition of hydroxylamine, nitrifier-denitrification and coupled nitrification-denitrification may also be involved in the production of N_2O, but their contribution is considered to be relatively small (Bremner, 1997; Butterbach-Bahl et al., 2013).

The production of N_2O in soils is affected by the presence of readily-available C fractions such as water-soluble organic C (Burford and Bremner, 1975), oxygen availability (Linn and Doran,1984), temperature (Saad and Conrad, 1993), pH (Bandibas et al., 1994), and the supply of ammonium and nitrate (Firestone and Davidson, 1989; Smith et al. 1997). As a result, it is easy to understand that any agricultural practice that causes changes in the soil N substrates or soil environmental conditions can lead to important variations in soil N_2O production.

Mineral N availability is a key process controlling soil N_2O fluxes. Bouwman et al. (2002) carried out a meta-analysis on 139 N_2O studies conducted in agricultural fields and observed an increase of N_2O emissions with increasing N application rates, mainly with application rates above 100 kg N ha^{-1}. Those results are also supported by the findings of Rees et al. (2013) who synthesized different European agricultural experiments. Earlier studies showed a greater amount of N_2O lost to the atmosphere when agricultural soils were manured than when mineral N fertilizers were used (Bouwman, 1990). However, other authors have obtained no differences between organic and mineral fertilizers (Meijide et al., 2009) or higher N_2O emissions when using mineral products (Meijide et al., 2007; Aguilera et al., 2013). Moreover, different results arise when separating between organic solid and liquid fertilizers (Aguilera et al., 2013). In their meta-analysis, the last authors found that only organic solid fertilizers led to significantly lower N_2O emissions than mineral fertilizers (Aguilera et al., 2013). Although in recent years several publications have covered the effect of fertilization on N_2O emission, much less attention has been paid to the interaction of different tillage and fertilization practices.

The use of conservation tillage practices has been claimed as a mechanism to reduce the CO_2 atmospheric pool by increasing the amount of organic carbon in the soil (Follett, 2001). Indeed, several studies have shown the benefits in terms of soil organic carbon sequestration when using no-tillage (NT) over a broad range of edaphoclimatic conditions (Follett, 2001). However, different authors have also suggested that the benefits obtained with the use of NT could be counterbalanced by an increase in N_2O emissions due to the greater amount of water in the soil and soluble forms of C in non-tilled systems (Aulakh et al., 1984; Ball et al., 1999; Smith et al., 2001). Nevertheless, Six et al. (2004) suggested that the emissions of N_2O could be reduced when maintaining NT over time. According to this last observation, van Kessel et al. (2013) conducted a meta-analysis on 239 direct comparisons between conventional tillage (CT) and NT and reduced tillage (RT) and pointed out that, on average, both NT and RT did not show greater N_2O emissions when compared with CT. However, they found a significant reduction in these emissions in long-term experiments (> 10 yr) under NT and RT practices, mainly in dry climates.

In some areas of semiarid Spain, a large livestock intensive farming sector is a relevant economic activity, resulting in high availability of manure. The application of organic fertilizers as amendments are a valuable resource for low-fertility soils and could lead to the increase in the amount of soil organic C and N (Hernández et al., 2013). In the Mediterranean area the use of RT or NT systems is increasingly adopted due to its agricultural and environmental benefits (Kassam et al., 2012). For instance, a better crop performance under NT due to greater soil water availability has been reported (Cantero-Martínez et al., 2003; Giambalvo et al., 2012). However, the interaction between the C input concomitant with the application of organic fertilizers and the greater amount of water stored in the soil usually found under NT in the Mediterranean agricultural systems could enhance the emission of N_2O to the atmosphere (Smith et al., 2001).

The objective of our study was to identify the optimum combination of tillage and N fertilization practices to reduce the amount of N_2O emitted from the soil to the atmosphere per unit of production in dryland Mediterranean areas. Our main hypotheses were that (i) due to the higher conservation of water under NT the emission of N_2O under this tillage system would be higher when compared with CT, (ii) the greater emissions under NT are compensated by a greater yield, and (iii) the combination of organic fertilizers and NT would increase the N emitted as N_2O due to the presence of labile C in the composition of the organic materials.

2. Material and Methods

2.1 Experimental sites

The study was carried out in two experimental fields with different tillage and fertilization management established in 1996 (long-term experiment) and 2010 (short-term experiment) in northeastern Spain. Selected site characteristics and soil properties for both experiments are detailed in Table 1.

In the long-term experiment, two types of tillage (NT, no-tillage, and CT, conventional intensive tillage) and three N fertilization rates (0, 60 and 120 kg N ha^{-1}) were compared. The CT treatment consisted of one pass of moldboard plow to 25 cm depth followed by two passes of a cultivator to 15 cm depth, both in September-October. Nitrogen fertilizer was applied manually and split into two applications: one-third of the rate before seeding as ammonium sulphate (21% N) and the rest at the beginning of tillering, in February, as ammonium nitrate (33.5% N). The cropping system consisted of continuous barley (*Hordeum vulgare L.,* cv. Hispanic from 1996 to 2010 and cv. Cierzo from 2010 to 2013). The historical management of the field prior to the establishment of the experiment was based on conventional intensive tillage with moldboard plowing and winter cereal monoculture.

In the short-term experiment, two tillage systems (CT with disk plow and NT), three N fertilization doses (0, 75 and 150 kg N ha^{-1}) and two types of N fertilizers (mineral N with ammonium sulphate and ammonium nitrate and organic N with pig slurry) were compared. In 2011, the CT treatment was carried out with two passes of chisel instead of disk plow due to the dry conditions of the soil. The treatment with 150 kg mineral N ha^{-1} was split into two manual applications, half of the dose before tillage as ammonium sulphate (21% N) and the other half at the beginning of tillering, in February, as ammonium nitrate (33.5% N),whereas the 75 kg N ha^{-1} treatment was applied entirely at tillering as ammonium nitrate. Similarly, in the pig slurry treatments, the 75 kg N ha^{-1} rate was applied entirely at tillering and the 150 kg N ha^{-1} one was split into two applications, one before tillage and the other one at tillering. The pig slurry was obtained from a commercial farm in the area and was conventionally surface-spread via a commercial vacuum tanker fitted with a splashplate, previously calibrated to apply the precise dose after analyzing the pig slurry composition. The cropping system before and during the experiment consisted of a barley (*Hordeum vulgare L.,* cv. Meseta) monoculture. Four years prior to the set-up of the experiment, soil management consisted of NT with mineral N fertilizer and application rates between 75 and 100 kg N ha^{-1}. Before that period passes of subsoiler and chisel were used since the 1970s.

For both experimental fields, the NT treatment included a total herbicide application (1.5 L 36% glyphosate per hectare) for controlling weeds before sowing. Planting was performed in November with a disk direct drilling machine set to 2-4 cm and harvesting was carried out with a commercial medium-sized combine in June. The straw residue was chopped and spread over the soil. Both experiments consisted of a randomized block design with three replications. Plot size in the long-term field experiment was 50 m x 6 m while in the short-term experiment plot size was 40 x 12 m in the organic N fertilization treatment and 40 x 6 m in the mineral N fertilization treatment. Also, for both experiments, air temperature and rainfall values were recorded hourly using an automated weather station located in each experimental area.

2.2 Soil N_2O emission quantification and analyses

The emission of N_2O from the soil to the atmosphere was measured with the non-steady-state chamber method (Hutchinson and Mosier, 1981). At the beginning of each experiment, two polyvinyl chloride rings (31.5 cm internal diameter) per plot were inserted into the soil to a depth of 5 cm. The rings were only removed at the time of tillage, planting and harvesting operations. Vented chambers of the same material and 20-cm height were fitted into the rings when the measurements were performed. The chambers were covered with a reflective insulation layer (model Aislatermic, Arelux, Zaragoza, Spain). A metal fitting was attached in the center of the top of the chamber and was lined with two silicon-Teflon septa as a sampling port.

Gas measurements were performed every two to three weeks. During the fertilizer applications more frequent samplings were performed (i.e., 24 h prior and 3 h and 72 h after fertilization). Each block of the experiment was assigned one operator in order to do the measurements for the different blocks simultaneously to reduce as much as possible the amount of time during the sampling process, thus avoiding temperature-induced biases (Rochette et al., 2012). Gas samples of 15 mL were obtained with polypropylene syringes at 0, 30 and 60 minutes after closing the chamber. Each sample was immediately injected into 12 mL Exetainer® borosilicate vials (model 038W, Labco, High Wycombe, UK). To quantify the amount of N_2O, gas samples were analyzed with an Agilent 7890A gas chromatography system equipped with an electrical conductivity detector (ECD) and an HP-Plot Q column (30 m long, 0.32 mm of section and 20 µm) with a pre-column 15 m long of the same characteristics. The injector and oven temperatures were set to 50ºC. The temperature of the ECD detector was set to 300 ºC, using a 5% methane in Argon gas mixture as a make-up gas at 30 mL min^{-1}. The system was calibrated using analytical grade standards (Carburos Metálicos, Barcelona, Spain). Soil N_2O emission rate was calculated taking into account the linear increase in the N_2O concentration within the chamber with time and correcting the values for the air temperature. The experiment covered three cropping seasons (2010-2011, 2011-2012 and 2012-2013) in the short-term experiment and two cropping seasons (2010-2011 and 2011-2012) in the long-term experiment. However, due to experimental constraints, gas samplings for both experiments began at the time of top-dressing application of fertilizers in February 2011.

2.3 Soil N-biomass input quantification

The quantification of the amount of N returned to the soil as above- and below-ground ground crop residues was performed in both experiments. Above-ground N biomass inputs were quantified in the 2011-2012 growing season in the long-term experiment and in the 2010-2011, 2011-2012 and 2012-2013 growing seasons in the short-term experiment by sampling plants along 0.5 m of the seeding line just before harvest, at three randomly selected locations per plot. Once in the laboratory the samples were dried at 65ºC during 48 h and threshed. Then all the plant but the grain (i.e., above-ground crop residues) was weighed. Root biomass was measured at flowering in April 2012 in the long-term and short-term experiments and in May 2013 in the short-term experiment. For each plot four soil cores (0-30 cm) were obtained, two in the seeding line and the other two between lines. Each soil sample was dispersed with a 5% sodium hexametaphosphate solution in a reciprocal shaker during at least 30 minutes and then washed by hand with a low-pressure shower jet through a 0.5-mm sieve to recover the roots, following the methodology proposed by Böhm (1979). Once washed, the sieve was submerged in a tray filled with water in order to ease collection of floating roots. Finally, root biomass was oven-dried at 65ºC and weighed. Root biomass per unit of area was calculated dividing the weight of roots by the area sampled with the core. Afterwards, above- and belowground biomass samples were analyzed for N content by dry combustion. The above- and below-ground biomass N inputs were calculated by multiplying the weight of each fraction by its N concentration. Grain yield of each treatment was measured in 2012 in the long-term experiment and in 2011, 2012 and 2013 in the short-term experiment by harvesting the plots with a commercial combine and weighing the grain.

2.4 Soil sampling and analyses

Soil samples from the 0-5 cm soil layer were obtained at every sampling date near (<1 m far) each gas sampling chamber for the determination of the water-filled pore space (WFPS) and the ammonium (NH_4^+) and nitrate (NO_3^-) present in the soil. The WFPS was obtained as the quotient between soil volumetric water content and total porosity. The volumetric water content was calculated as the gravimetric water content times the soil bulk density. The gravimetric water content was obtained by oven drying the soil samples at 105 ºC for the long-term experiment and at 50ºC for the short-term experiment until constant weight. In the short-term experiment, soil was dried at 50ºC in order to avoid the dehydration of the gypsum present in the soil of this experiment (Porta, 1998). Soil porosity was calculated as a function of soil bulk density assuming a particle density of 2.65 Mg m^{-3}. In turn, soil bulk density was determined with the cylinder method. The soil NH_4^+ and NO_3^- contents were calculated by extracting 50 g of fresh soil with 100 mL of 1M KCl. The extracts were analyzed with a continuous flow autoanalizer (Seal Autoanalyzer 3, Seal Analytical, Norderstedt, Germany). Both ions were transformed to kg N ha^{-1} in a dry soil basis taking into account soil moisture and bulk density.

Also, soil total nitrogen (STN) stocks were calculated in June 2013 in the short-term experiment. To this end, soil samples were taken from five depths (0-5, 5-10, 10-25, 25-50 and 50-75 cm) at two selected areas per plot. For the same depths soil bulk density was determined using the cylinder method. Afterwards samples were air-dried and 0.5-mm sieved. The STN concentration was determined by dry combustion. Finally, the concentration values were transformed to STN stocks following the equivalent soil mass procedure (Ellert and Bettany, 1995).

2.5 Total N loss as N_2O, yield-scaled N_2O emission and statistical analysis

Total N loss as N_2O during the entire period of gas measurements (i.e from February 2011 to August 2012 in the long-term experiment and from February 2011 to June 2013 in the short-term experiment) was quantified with the trapezoid rule. In the long-term experiment, the yield-scaled N_2O ratio, expressed as kg of CO_2 equivalents emitted per kg of grain produced, was calculated for the 2011-2012 growing season by integrating the N_2O fluxes from the pre-seeding application of fertilizers (October 2011) until harvest (June 2012), taking into account that the GWP for the N_2O is 298 times higher than that of CO_2 (Forster et al., 2007) and dividing that result by the amount of grain produced by each treatment in that cropping season. In the short-term experiment, the ratio was calculated for two growing seasons (2011-2012 and 2012-2013) by integrating the emissions of N_2O from the pre-seeding application of fertilizers in the 2011-12 growing season (October 2011) until the harvest of the 2012-13 season (June 2013) and dividing that result by the sum of grain produced by each treatment in both cropping seasons.

Data for N_2O fluxes, soil ammonium and nitrate content and WFPS were analyzed using the JMP 10 statistical package (SAS Institute Inc, 2012) performing a repeated measures analysis of variance (ANOVA) with tillage, N fertilization, date of sampling and their interactions as sources of variation. When needed, a Box-Cox data transformation was used in order to normalize the data and the variances. Also, different ANOVA were performed for cumulative N loss, above- and below-ground biomass N inputs, STN stocks and yield-scaled N_2O ratio with tillage, N fertilization and their interaction as sources of variation. When significant, differences among treatments were identified at 0.05 probability level of significance using a Tukey test. The statistical package JMP 10 (SAS Institute Inc., 2012) was also used to test the presence of linear relationships between N_2O fluxes and soil ammonium, nitrate, WFPS and temperature at 0-5 cm soil depth.

3. Results

3.1 Weather conditions during the experimental period

Air temperature and precipitation in the long- and short-term experiments are presented in Table 2. Given the average annual precipitation at the long-term (430 mm) and the short-term (327 mm) experimental sites (Table 1), the 2010-2011 and 2011-2012 growing seasons could be considered drier than average in both experiments. In contrast, the 2012-2013 growing season registered a higher rainfall than the average in the short-term experiment, with a total of 537 mm (Table 2). Throughout the experimental period, the air temperature showed the typical pattern of the Mediterranean region with hot summers and cold winters.

3.2 Tillage effects

3.2.1. WFPS, soil ammonium and soil nitrate content

In the long-term experiment, the WFPS only exceeded the value of 65% in two of all the samplings performed whereas in the short-term experiment WFPS values were below that threshold during the whole experimental period (data not shown). Tillage affected significantly to WFPS with the NT treatment presenting the highest values in most samplings dates (data not shown). In both experiments, the mean soil ammonium values were low (<2 kg NH_4^+-N ha^{-1}) (Tables 3 and 4). However, as shown in Figs. 1A and 1C, the application of fertilizers led to a fast and short-lived increase in soil ammonium content. Significant differences between tillage treatments in soil ammonium content were only found in the long-term experiment (Fig. 1A). Soil nitrate content in the soil surface (0-5 cm) was affected by tillage, with higher mean values under CT (56.4 kg NO_3^--N ha^{-1}) when compared with NT (36.1 kg NO_3^--N ha^{-1}) (Table 3). In the long-term experiment, a greater accumulation of nitrate in the soil was observed under CT compared with NT during the last months studied (February-August 2012) (Fig. 1B). In the case of the short-term experiment (Fig. 1D), tillage treatments presented similar nitrate content in the soil.

3.2.2. Soil N_2O emission, N-biomass inputs and yield-scaled N_2O emission

In the long-term experiment, the average N_2O flux throughout the entire experimental period was 0.137 and 0.141 mg N_2O-N m^{-2} d^{-1} for the CT and NT treatments, respectively, without significant differences between them (Table 3). Contrarily, in the short-term experiment, a greater average N_2O flux was observed under NT (0.205 mg N_2O-N m^{-2} d^{-1}) than under CT (0.139 mg N_2O-N m^{-2} d^{-1}). In the long-term experiment, N_2O fluxes ranged from -0.09 to1.14 mg N_2O-N m^{-2} d^{-1} while in the short-term experiment the fluxes varied from -0.05 to 0.91 mg N_2O-N m^{-2} d^{-1} (Fig. 2). Tillage treatments presented significant differences for six sampling dates in the long-term experiment in which, half of the dates, CT presented the highest values and in the other half NT (Fig. 2). In the short-term experiment, significant differences were found only in four sampling dates, being always NT the highest N_2O emitter. When integrating all the sampling period neither the long-term experiment nor the short-term experiment showed significant differences between tillage systems in cumulative N losses as N_2O (Tables 2 and 3).

In both the long-term and short-term experiments, the use of NT significantly increased the production of grain (Tables 3 and 4). Moreover, NT increased aboveground soil N input in both experiments, but not belowground N input (Tables 3 and 4).

When cumulative N-N_2O losses were related to grain yield, in both experiments NT significantly reduced yield-scaled N_2O emissions compared with CT. In the long-term experiment, the CT treatment emitted 0.362 kg CO_2 eq. kg^{-1} grain while the NT treatment emitted 0.033 kg CO_2 eq. kg^{-1} grain (Table 3). In the case of the short-term experiment, CT and NT averaged 0.104 and 0.056 kg of CO_2 eq. emitted per kg of grain, respectively (Table 4).

3.3 Nitrogen fertilizer type and rate effects

3.3.1. Soil ammonium and nitrate content

In the long-term experiment, the application of 60 and 120 kg N ha^{-1} increased soil ammonium contents compared to the control treatment (Fig. 3A). On the other hand, the increasing N rates in the long-term experiment (0, 60 and 120 kg N ha^{-1}) were accompanied by increasing amounts of nitrate in the soil surface (0-5 cm): 19.1, 50.0 and 69.8 kg NO_3^--N ha^{-1}, respectively (Table 3). Differences between N fertilization rates were found in the dynamics of soil nitrate content (Fig. 3B), with the highest values under the 120 kg N ha^{-1} treatment for most of the sampling dates and the lowest in the control treatment. In the short-term experiment the application of fertilizers was accompanied by large increases in the amount of ammonium in the soil (Table 4 and Fig. 3C). Significant differences were found between N fertilization treatments for the nitrate content at the soil surface layer (0-5 cm), with the greater values under the treatment with 150 kg mineral N ha^{-1} (Fig. 3D).

3.3.2. Soil N_2O emission, N-biomass inputs and yield-scaled N_2O emission

The average N_2O fluxes quantified during the gas measurement period in the long-term experiment were 0.113, 0.122 and 0.181 mg N_2O-N m^{-2} d^{-1} for the 0, 60 and 120 kg mineral N ha^{-1} N rates, respectively, without significant differences between treatments (Table 3). In the short-term experiment the lowest fluxes were observed in the control treatment (0.102 mg N_2O-N m^{-2} d^{-1}) and the highest when 150 kg mineral N ha^{-1} were applied (0.271 mg N_2O-N m^{-2} d^{-1}), with a higher emission of N_2O when increasing the amount of N applied to the soil for both mineral and organic fertilizers. However, no differences were found between N types (mineral or organic) on the average N_2O flux for a given N rate (Table 4). In both experiments, significant differences between N rates were observed in four sampling dates (Fig. 4). Furthermore, for both experiments low negative fluxes were also found in different sampling dates.

The increasing rates of mineral N fertilizer (0, 60 and 120 kg N ha^{-1}) significantly increased the production of grain in the long-term experiment (720, 941 and 1040 kg grain ha^{-1}, respectively) (Table 3). In contrast, in the short-term experiment only the highest rate of mineral N (150 kg N ha^{-1}) showed greater grain yield that the control (0 kg N ha^{-1}) and the medium rate (75 kg N ha^{-1}) (Table 4). The use of pig slurry as a fertilizer increased the grain production compared with the mineral fertilizer and also showed a significant increase in yield (sum of the grain produced in the 2011-2012 and 2012-2013 growing seasons) when increasing the rate of slurry applied (4657 and 5335 kg grain ha^{-1} for the application of 75 and 150 kg organic N ha^{-1}, respectively) (Table 4).

The above-ground N input to the soil in the long-term experiment was 3.33, 4.84 and 5.46 g N m^{-2} for the 0, 60 and 120 kg N ha^{-1} treatments, respectively, with significant differences between them (Table 3). In turn, the same parameter in the short-term experiment reached 3.1, 4.5, 4.9, 3.3 and 5.6 g N m^{-2} for the control, the 75 and 150 kg mineral N ha^{-1} and the 75 and 150 kg organic N ha^{-1} treatments, respectively, with significant differences between them (Table 4). The input of N to the soil due to below-ground biomass did not show differences between N fertilization practices neither in the long-term experiment nor in the short-term experiment (Tables 3 and 4).

Differences between N fertilization treatments in yield-scaled emissions of N_2O were only found in the short-term experiment. The ratio increased when the mineral N rate increased (0.075, 0.096 and 0.131 kg CO_2 eq. kg^{-1} grain for the 0, 75 and 150 kg mineral N ha^{-1}, respectively). Furthermore, the application of pig slurry showed the lowest ratio (for both 75 and 150 kg N ha^{-1} rates) (Table 4).

3.4 Tillage and nitrogen interaction

In the short-term experiment, the interaction between tillage and N fertilization was significant for N_2O fluxes, above-ground N inputs and grain yield (Table 4). In the case of the long-term field experiment, the interaction between tillage and N fertilization rates was only significant for soil surface NO_3^- and grain yield (Table 3).

In the long-term experiment, no differences in grain yield were found between N rates under CT. However, under NT greater grain yield was observed when 60 and 120 kg N ha^{-1} were applied in comparison with the control treatment (Table 3). Contrarily, in the short-term experiment, in NT the application of mineral and organic N at different rates did not lead to significant differences in grain yield but in CT, for a given N rate, the use of pig slurry increased the production of grain when compared with the use of mineral N (Table 4).

In the case of the above-ground N inputs to the soil as crop residues, in the short-term experiment, CT did not show differences between N fertilization treatments. However, in NT greater above-ground N inputs were quantified when applying 75 and 150 kg mineral N ha^{-1} and 150 kg N ha^{-1} of pig slurry when compared to the control treatment (Table 4).

3.5. Soil total N (STN) stocks

In the short-term experiment, STN stocks for the whole soil profile (0-75 cm) ranged from 5.2 to 8.9 for NT without N (control treatment) and CT with 75 kg organic N ha^{-1}, respectively (data not shown). However, after three years of contrasting treatments no significant differences between combinations of tillage and N fertilization were observed in the stocks neither in the soil surface (0-10 cm) nor in the entire soil profile (0-75 cm).

4. Discussion

4.1 N_2O fluxes range and production mechanisms

In our study, most of the N_2O fluxes measured were within the range found in other similar studies performed in Mediterranean conditions (Meijide et al., 2009; Aguilera et al., 2013). In addition, WFPS values were below 65%. According to the findings of Linn and Doran (1984), this result would suggest that nitrification was the main process producing N_2O and that denitrification was restricted to anaerobic microsites due to the O_2 inhibitory effect on this process (Sexstone et al., 1985). In a fertilizer study conducted in a rainfed field experiment in Spain, Meijide et al. (2009) pointed out that denitrification was the N_2O producing process for only a few days during their experiment, on the basis of their denitrification estimates using the acetylene technique.

The low fluxes N_2O fluxes observed in our study were accompanied by several episodes of net N_2O uptake. Soil consumption of N_2O has been measured under various edaphoclimatic conditions (Chapuis-Lardy et al., 2007). Although this process is usually related to the complete denitrification of N_2O to N_2 as a consequence of low soil mineral N and high water contents, recently its occurrence has also been reported in dry, oxic soils (Wu et al., 2013; Rees et al., 2013). Aerobic denitrification is assumed to be one of the main processes for N_2O consumption in dry soils with high O_2 concentration (Bateman and Baggs, 2005).

No significant linear relationships were observed between the fluxes of N_2O and the different variables measured in any of the two experimental fields (data not shown). However, the increase in the concentration of ammonium in the soil when mineral and organic fertilizers were applied was followed by an increase in the magnitude of the fluxes of N_2O to the atmosphere, mainly when NT was used. This result would be explained by the greater amount of water in under NT that would lead to a fast nitrification of the ammonium to nitrate, resulting in significant pulses of N_2O. The quantification of the NO/N_2O ratio has been pointed out as a useful means to identify the mechanism of production of N oxides (Meijide et al. 2009). With the use of that ratio, the last authors concluded that the nitrification of ammonium applied to the soil right after the fertilization was the main N_2O production mechanism when applying nitrogen in dryland Mediterranean soils. In turn, the long-term accumulation of nitrate in the soil when CT and high N rates are used would act as a substrate for the production of N_2O through the denitrification process. The lack of N consumption by the crop and episodic periods of intense rainfall during the summer months (i.e. summer 2011 in the short-term experiment) in the Mediterranean area can exacerbate the loss of N as N_2O.

4.2 Tillage effects

Several studies have shown greater emission of N_2O under NT than under CT (Skiba et al., 2002; Ball et al., 1999). This finding is usually related to a higher level of aeration and higher gas diffusivity when the soil is tilled. However, other authors have suggested lower N_2O emissions when NT is practiced for a long-term (> 10 years) (Omonode et al., 2011; Van Kessel et al., 2013) or in well aerated soils (Rochette, 2008). In our study, tillage significantly affected the average emissions of N_2O in the short-term experiment, with greater values under NT than under CT. In contrast, in the long-term experiment, the mean N_2O fluxes were the same for both tillage treatments. The different N_2O emission pattern observed between the short-term and the long-term experiments could possibly be due to an improvement of soil structure under long-term NT. This improvement would be related to the increase in soil organic matter content in soil surface (0-10 cm) under NT in the long-term experiment when compared to CT that we found in previous works (Morell et al., 2011; Plaza-Bonilla et al. 2014). In the same experimental site we observed an increase in the proportion of soil water-stable macroaggregates when NT was maintained over time (Plaza-Bonilla et al., 2013). Thus, the greater amount of soil organic matter and the increase of macroaggregate water-stability under NT would have led to a reduction of anaerobic microsites and improved soil gas diffusivity diminishing the conditions for N_2O production by denitrification (van Kessel et al., 2013). Moreover, the lower amount of nitrate found in NT compared with CT in the long-term experiment could also have impacted N_2O production and fluxes due to a lower amount of N available for denitrification.

The use of NT greatly reduced the yield-scaled N_2O emissions compared with CT regardless the number of years of the experiment. In rainfed Mediterranean agrosystems, the occurrence of terminal drought during the grain filling period usually leads to a reduction in potential yield (Loss and Siddique, 1994; González et al., 2007). In these conditions, the use of NT increases the amount of water stored in the soil leading to a greater production of above-ground biomass and grain yield, mainly in dry years (Cantero-Martínez et al., 2003). This fact is also demonstrated in our results since above-ground N inputs and grain yield were higher in NT compared with CT, thus reducing the emissions of N_2O as CO_2 equivalents per kg of grain produced. These results are in line with those of Van Groenigen et al. (2010), who concluded that N uptake must be maximized in order to minimize N_2O emissions while maintaining or increasing crop yield.

4.3 Nitrogen type and rate effects

The application of increasing rates of mineral N fertilizer led to higher emissions of N_2O, although those emissions were only significantly different in the short-term experiment. Different authors reported a positive relationship between N_2O emissions and soil mineral N content and N application rates (Bouwman, 1996; Bouwman et al., 2002; Halvorson et al., 2008). In turn, in other studies it has been observed that when the threshold for satisfying crop N needs is exceeded, N_2O emissions increase dramatically (Snyder et al., 2009; van Groenigen et al. 2010). In the area of our experiments, Angás et al. (2006) observed higher nitrogen use efficiency at lower N rates in a tillage and N application rate study. Their results would explain the greater N_2O-N losses we found when increasing the amount of mineral N applied to the soil. However, although the application of mineral N led to an increase of grain yield, this increase was not enough to counterbalance the increase in N_2O emissions resulting in the lack of differences between mineral N rates on yield-scaled N_2O emissions.

We found no differences in N_2O fluxes between fertilizer types. In our study, we used mineral N and pig slurry, because these are the most common fertilizers used in the area. It is known that pig slurry tends to show a similar behavior to mineral fertilizers due to its high NH_4^+ content (Sánchez-Martín et al., 2010), which can be rapidly nitrified in well aerated soils. Some studies have suggested, however, that liquid manure can activate the denitrifying soil microbial community due to the readily oxidizable C and sufficient mineralizable N they provide (Johnson et al., 2007). But in dryland agrosystems the addition of C compounds through pig slurry could mainly affect NO fluxes whereas in irrigated or more humid systems they could merely stimulate N_2O emissions (Meijide et al., 2009).

The use of 75 and 150 kg N ha^{-1} as pig slurry led to a grain yield 28% and 37% higher than the equivalent rate of mineral N, respectively. The lack of differences in above-ground N inputs between both types of fertilizers could be explained by a better allocation of resources for grain production when using pig slurry. The increase in grain yield with pig slurry led to a 2 to 2.5 times reduction in the yield-scaled N_2O emissions. Our results agree with those of Hernández et al. (2013) who compared the response of barley crop to the application of mineral fertilizer and different rates of pig slurry in a rainfed area of Spain and found a positive effect of the pig slurry on grain yield. Similarly, Thomsen and Sørensen (2006) also found an enhanced crop N uptake when using pig slurry compared with mineral fertilizer.

4.4 Tillage and nitrogen interaction effects

In the long-term experiment, the interaction between tillage and mineral N fertilization rates affected significantly the production of grain. Under NT the application of 60 kg N ha^{-1} was followed by an increase in grain production, whereas under CT there were no differences in grain yield. In semiarid Mediterranean agroecosystems, water is the most limiting factor for crop production and the response to N application depends on soil water availability (Cantero-Martínez et al., 1995). Consequently, tillage systems that maintain a higher amount of water in the soil, such as NT, usually lead to a better crop response to N application (Angás et al., 2006; Morell et al., 2011).

In the short-term experiment, a significant interaction between tillage and fertilizer type was found. In this case, whereas the different types and rates of N did not produce a significant increase in crop yield under NT, the application of pig slurry under CT significantly improved grain production compared with mineral N fertilization. Maltas et al. (2013) compared the production of different crops when using mineral and organic fertilizers in a 12-yr field experiment under a reduced tillage management similar to that in our long-term experiment. They observed a significantly higher grain yield when pig slurry was applied and suggested that this positive effect of slurry could be due to a more diversified mineral fertilization provided by animal manures.

5. Conclusions

Our results demonstrate that under rainfed Mediterranean conditions tillage significantly affected the loss of N as N_2O emitted to the atmosphere, with higher N_2O emissions under NT in the short-term (<4 years). However, when NT was used in the long-term (>10 years), N_2O fluxes were similar than under CT. The application of N fertilizers increased N_2O emissions mainly when high rates of mineral N were used and particularly immediately after the application of fertilizers.

Although the use of mineral and organic fertilizer resulted in similar N_2O fluxes, a significant increase in grain yield using pig slurry resulted in lower yield-scaled N_2O emissions. Likewise, in the two field experiments reported in this study, the use of NT significantly reduced the yield-scaled emissions of N_2O due to better crop performance. Therefore, we can conclude that in rainfed Mediterranean agroecosystems, the use of NT and pig slurry as N fertilizer is an efficient management practice for reducing the amount of N_2O emitted per kg of grain produced.

Acknowledgements

We would like to express our gratitude to Montse Llovera, Ana Bielsa, Javier Bareche, Silvia Martí, Carlos Cortés, Josan Palacio, Héctor Martínez, Miquèl Segalàs, Jonatan Ovejero, Douglas Winter, Soni Hueftle and Dr. Jorge Lampurlanés for their help at different stages of this study. D. Plaza-Bonilla was awarded with a FPU fellowship by the Spanish Ministry of Education. This research was supported by the Comisión Interministerial de Ciencia y Tecnología of Spain (grants AGL 2007-66320-C02-01 and AGL 2010-22050-C03-01/02), the Aragon Government and La Caixa (grant GA-LC-050/2011), the Department of Agriculture of the Catalonia Government (grant 2012 AGEC 00012) and the European Union (FEDER funds).

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Figure captions

Fig. 1 Soil ammonium (A) and nitrate (B) content in the long-term experiment and ammonium (C) and nitrate (D) content in the short-term experiment in the 0-5 cm soil layer as affected by tillage (CT, conventional tillage; NT, no-tillage). * Indicates significant differences between tillage treatments for each date and experiment at $P<0.05$. Vertical arrows indicate fertilizer applications.

Fig. 2 Soil N_2O fluxes in the long- and short-term experiments as affected by tillage (CT, conventional tillage; NT, no-tillage). * Indicates significant differences between tillage treatments for each date and experiment at $P<0.05$. Vertical arrows indicate fertilizer applications.

Fig. 3 Soil ammonium (A) and nitrate (B) content in the long-term experiment and ammonium (C) and nitrate (D) content in the short-term experiment in the 0-5 cm soil layer as affected by N fertilization treatment (long-term experiment: 0, 60 and 120 kg mineral N ha^{-1}; short-term experiment: 0, control; 75 Min, 75 kg mineral N ha^{-1}; 150 Min, 150 kg mineral N ha^{-1}; 75 Org, 75 kg organic N ha^{-1} with pig slurry; 150 Org, 150 kg organic N ha^{-1} with pig slurry). * and different lower-case letters indicate significant differences between N fertilization treatments for each date and experiment at $P<0.05$. Vertical arrows indicate fertilizer applications.

Fig. 4 Soil N_2O fluxes in the long- and short-term experiments as affected by N fertilization treatment (long-term experiment: 0, 60 and 120 kg mineral N ha^{-1}; short-term experiment: 0, control; 75 Min, 75 kg mineral N ha^{-1}; 150 Min, 150 kg mineral N ha^{-1}; 75 Org, 75 kg organic N ha^{-1} with pig slurry; 150 Org, 150 kg organic N ha^{-1} with pig slurry). Different lower-case letters indicate significant differences between N fertilization treatments for each date and experiment at $P<0.05$. Vertical arrows indicate fertilizer applications.

Table 1. General site and soil characteristics in the 0- to 30-cm soil depth at the beginning of the experiments at the two study sites.

Site and soil characteristic	Long-term experiment	Short-term experiment
Year of establishment	1996	2010
Latitude	41º 48’ 36’’ N	41º 54’ 12’’ N
Longitude	1º 07’ 06’’ E	0º 30’ 15’’ W
Elevation, m	330	395
Annual precipitation, mm	430	327
Mean annual air temperature, ºC	13.8	13.4
Annual ETo, mm	855	1197
Soil classification†	Typic Xerofluvent	Typic Calcixerept
pH (H_2O, 1:2.5)	8.5	8.0
$EC_{1,5}$, dS m^{-1}	0.15	1.04
Organic C, g kg^{-1}	7.6	15.6
Organic N, g kg^{-1}	-	1.4
Particle size distribution, %		
Sand (2000-50 µm)	46.5	6.2
Silt (50-2 µm)	41.7	63.3
Clay (<2 µm)	11.8	30.5

† According to the USDA classification (Soil Survey Staff, 1994).

Table 2. Mean monthly air temperature (T) and monthly precipitation (P) in the short-term and long-term field experiments during the 2010-2011, 2011- 2012 and 2012- 2013 growing seasons.

	Long-term experiment				Short-term experiment					
Month	2010-2011		2011-2012		2010-2011		2011-2012		2012-2013	
	T	P	T	P	T	P	T	P	T	P
July	25.4	5	22.6	19	24.7	7.4	22.1	6	23.5	2
August	23.2	16	24.9	29	22.9	0.5	23.9	4.6	25.6	7
September	18.5	21	22.0	2	17.7	37.3	20.6	8.9	20.2	18
October	12.9	1	16.5	22	12.1	57.5	14.5	32.4	15.5	212
November	6.2	0	10.9	82	6.9	38.8	10.2	45.4	10.1	27
December	3.2	0	5.7	2	2.9	21.4	7.1	4.9	7.7	16
January	2.7	0	4.3	4	3.6	19.4	6.9	0	7.1	27
February	5.3	12	3.6	2	6.8	13.3	5.5	1	6.8	6
March	8.7	36	11.3	5	8.9	90.5	12.0	17	10.0	85
April	14.3	20	11.8	62	14.5	16.2	11.7	105	12.4	66
May	18.2	27	18.3	19	17.8	35.5	18.3	8	13.4	18
June	20.6	73	23.8	12	20.6	0.7	23.0	47	19.1	53
Year	13.3	211	14.7	260	13.3	338.5	14.6	280.2	14.3	537

Table 3. Analysis of variance of nitrate and ammonium content in the soil (0-5 cm) (kg NO_3^--N ha^{-1} and kg NH_4^+-N ha^{-1}, respectively), above- and below-ground N-biomass inputs, N_2O flux (mg N_2O-N m^{-2} d^{-1}), cumulative N-loss during the whole experimental period (g N ha^{-1}), 2011-12 grain yield (kg ha^{-1} at 10% moisture) and the ratio between the loss of N_2O in CO_2 equivalents and the grain yield of the 2011-12 growing season as affected by tillage (CT, conventional tillage; NT, no-tillage), N fertilization treatments (0, 60 and 120 kg mineral N ha^{-1}), date of sampling (growing season in the case of N-biomass inputs) and their interactions in the long-term field experiment.

	Long-term experiment							
Treatments	Soil nitrate (0-5 cm)	Soil ammonium (0-5 cm)	N-biomass input (g N m^{-2})		N_2O flux	Cumulative N-loss	2011-2012 Grain yield	kg CO_2 eq kg grain^{-1}
			Above-ground	Below-ground				
CT	56.40 a¶	8.59	2.11 b	1.21	0.137	616	245.8 b	0.362 a
NT	36.07 b	9.59	6.97 a	1.49	0.141	549	1554.3 a	0.033 b
0	19.11 c	3.44 b	3.33 b	1.16	0.113	473	719.6 c	0.095
60	50.02 b	10.69 a	4.84 ab	1.67	0.122	532	940.7 b	0.290
120	69.82 a	13.15 a	5.46 a	1.21	0.181	742	1039.8 a	0.208
CT – 0	21.68	3.49	1.88	1.14	0.100	442	178.4 c	0.170
CT – 60	60.07	8.70	2.22	1.58	0.153	681	226.8 c	0.563
CT – 120	87.46	13.57	2.24	0.90	0.158	724	332.1 c	0.354
NT – 0	16.53	3.39	4.78	1.18	0.126	503	1260.8 b	0.019
NT- 60	39.98	12.69	7.45	1.76	0.093	383	1654.5 a	0.018
NT – 120	51.93	12.72	8.69	1.52	0.205	759	1747.5 a	0.062
			ANOVA					
Tillage	*0.004*	*0.476*	*<0.001*	*0.167*	*0.817*	*0.591*	*<0.001*	*<0.001*
Nitrogen	*<0.001*	*<0.001*	*0.047*	*0.098*	*0.094*	*0.191*	*<0.001*	*0.164*
Tillage x Nitrogen	*0.163*	*0.275*	*0.452*	*0.461*	*0.290*	*0.425*	*<0.001*	*0.155*
Date	*<0.001*	*<0.001*			*<0.001*			
Tillage x Date	*<0.001*	*<0.001*			*<0.001*			
Nitrogen x Date	*<0.001*	*<0.001*			*<0.001*			
Tillage x Nitrogen x Date	*<0.001*	*0.340*			*0.121*			

¶ For a given variable, different lower-case letters indicate significant differences between treatments at $P<0.05$.

Table 4. Analysis of variance of nitrate and ammonium content in the soil (0-5 cm) (kg NO_3^--N ha^{-1} and kg NH_4^+-N ha^{-1}, respectively), above- and below-ground N-biomass inputs, N_2O flux (mg N_2O-N m^{-2} d^{-1}), cumulative N-loss during the whole experimental period (g N ha^{-1}), 2011-2012 plus 2012-2013 grain yield (kg ha^{-1} at 10% moisture) and the ratio between the loss of N_2O in CO_2 equivalents and the production of grain of the 2011-2012 plus 2012-13 growing seasons as affected by tillage (CT, conventional tillage; NT, no-tillage), N fertilization treatments (0, control; 75 Min, mineral N at 75 kg N ha^{-1}; 150 Min, mineral N at 150 kg N ha^{-1}; 75 Org, organic N with pig slurry at 75 kg N ha^{-1} and 150 Org, organic N with pig slurry at 150 kg N ha^{-1}), date of sampling (growing season in the case of N-biomass inputs) and their interactions in the short-term field experiment.

Treatments	Short-term experiment							
	Soil nitrate (0-5 cm)	Soil ammonium (0-5 cm)	N-biomass input (g N m^{-2})		N_2O flux	Cumulative N-loss	2011-13 Grain yield	kg CO_2 eq kg grain^{-1}
			Above-ground	Below-ground				
CT	98.18	11.43	3.50 b	1.18	0.139 b	971	2262.8 b	0.104 a
NT	96.01	10.86	5.09 a	1.43	0.205 a	1311	5692.3 a	0.056 b
0	64.78 bc¶	2.86 c	3.13 c	1.05	0.102 c	700 b	2358.6 d	0.075 ab
75 Min	106.53 ab	8.69 b	4.51 ab	1.00	0.132 bc	911 b	3651.1 c	0.096 ab
150 Min	148.29 a	15.95a	4.86 ab	1.52	0.271 a	1804 a	3885.2 c	0.131 a
75 Org	59.11 c	12.29 ab	3.34 bc	1.79	0.143 bc	1007 ab	4657.4 b	0.048 b
150 Org	106.78 ab	15.88 a	5.64 a	1.13	0.213 ab	1283 ab	5335.4 a	0.051 b
CT – 0	57.02	3.11	3.30 cd	0.89	0.088	624	992.3 f	0.099 ab
CT – 75 Min	116.54	8.40	2.58 d	0.53	0.098	647	1308.3 ef	0.144 ab
CT – 150 Min	153.67	17.54	4.60 bcd	1.35	0.279	1847	2225.7 de	0.179 a
CT – 75 Org	68.29	10.79	3.02 cd	1.88	0.106	736	2755.4 cd	0.052 b
CT- 150 Org	95.40	17.30	4.00 bcd	1.14	0.125	1001	4032.6 b	0.048 b
NT – 0	72.54	2.62	2.96 cd	1.21	0.117	776	3725.0 bc	0.051 b
NT – 75 Min	96.52	8.97	6.44 ab	1.46	0.166	1175	5993.9 a	0.048 b
NT – 150 Min	142.91	14.35	5.12 abc	1.68	0.264	1761	5544.8 a	0.083 ab
NT – 75 Org	49.92	13.79	3.65 cd	1.68	0.178	1278	6559.5 a	0.043 b
NT- 150 Org	118.16	14.46	7.28 a	1.12	0.299	1565	6638.3 a	0.055 b
			ANOVA					
Tillage	*0.820*	*0.577*	*<0.001*	*0.225*	*0.011*	*0.069*	*<0.001*	*0.004*
Nitrogen	*<0.001*	*<0.001*	*<0.001*	*0.058*	*<0.001*	*0.004*	*<0.001*	*0.009*
Tillage x Nitrogen	*0.482*	*0.298*	*<0.001*	*0.547*	*0.164*	*0.726*	*<0.001*	*0.136*
Date	*<0.001*	*<0.001*	*<0.001*	*0.093*	*<0.001*			
Tillage x Date	*<0.001*	*0.512*	*0.483*	*0.209*	*0.002*			
Nitrogen x Date	*<0.001*	*<0.001*	*0.074*	*0.325*	*<0.001*			
Tillage x Nitrogen x Date	*0.717*	*0.732*	*0.006*	*0.443*	*0.003*			

¶ For a given variable, different lower-case letters indicate significant differences between treatments at $P<0.05$.

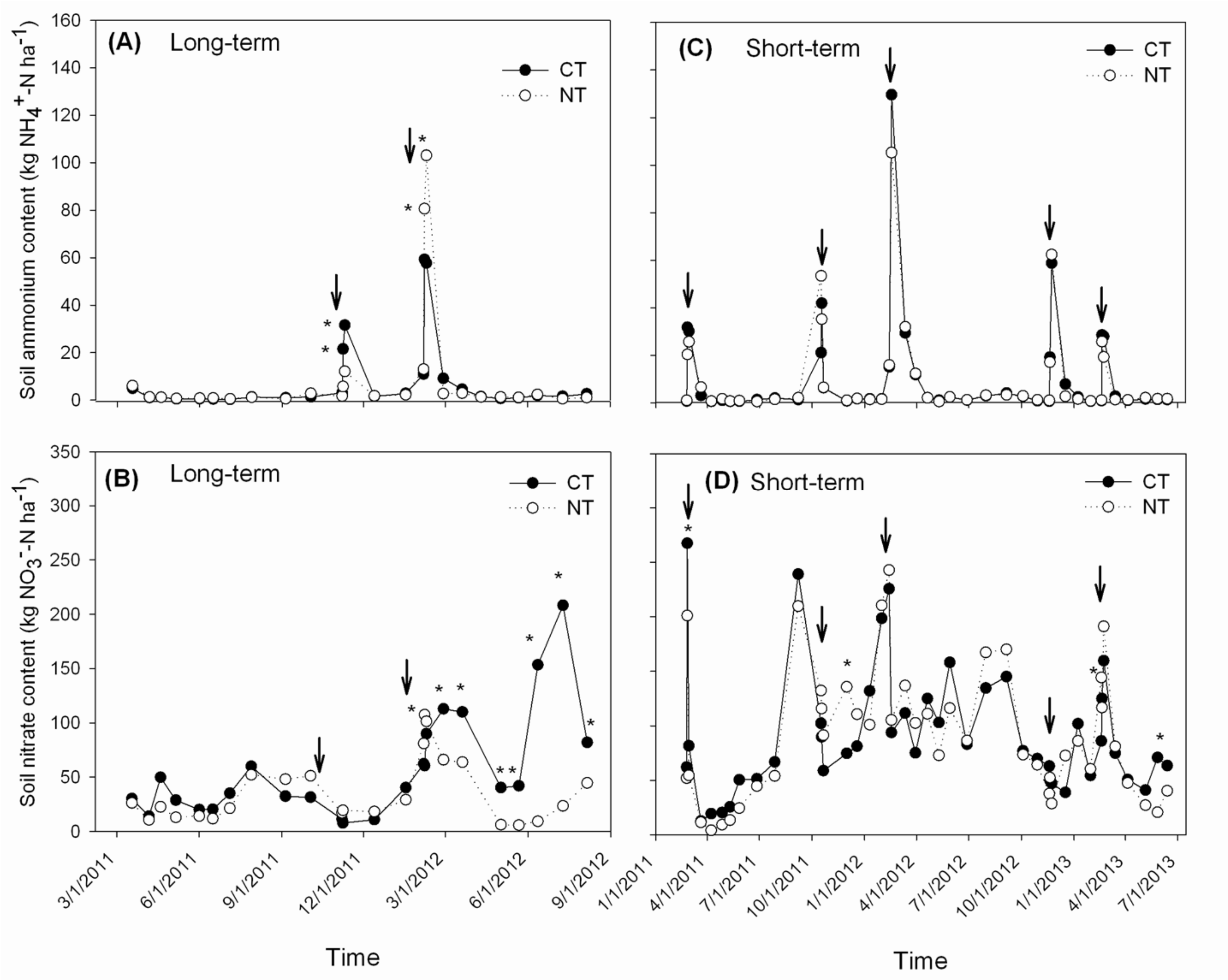

(A) Long-term
(B) Long-term
(C) Short-term
(D) Short-term
CT
NT
Soil ammonium content (kg NH_4^+-N ha^{-1})
Soil nitrate content (kg NO_3^--N ha^{-1})
0
20
40
60
80
100
120
140
160
50
150
200
250
300
350
*
3/1/2011
6/1/2011
9/1/2011
12/1/2011
3/1/2012
6/1/2012
9/1/2012
1/1/2011
4/1/2011
7/1/2011
10/1/2011
1/1/2012
4/1/2012
7/1/2012
10/1/2012
1/1/2013
4/1/2013
7/1/2013
Time

Fig. 1

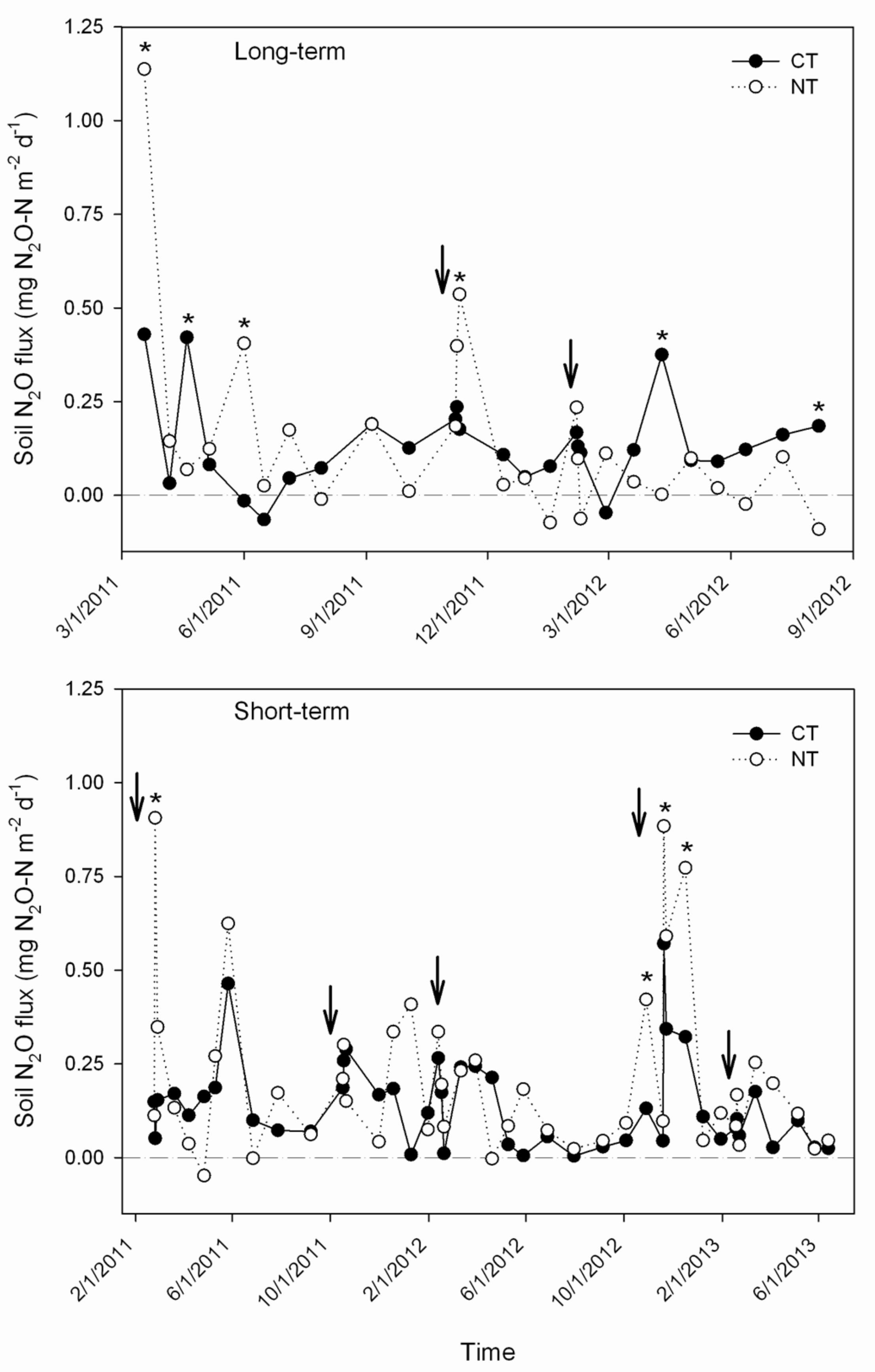

Long-term
CT
NT
Soil N$_2$O flux (mg N$_2$O-N m^{-2} d^{-1})
1.25
1.00
0.75
0.50
0.25
0.00
3/1/2011
6/1/2011
9/1/2011
12/1/2011
3/1/2012
6/1/2012
9/1/2012
Short-term
CT
NT
Soil N$_2$O flux (mg N$_2$O-N m^{-2} d^{-1})
1.25
1.00
0.75
0.50
0.25
0.00
2/1/2011
6/1/2011
10/1/2011
2/1/2012
6/1/2012
10/1/2012
2/1/2013
6/1/2013
Time

Fig. 2

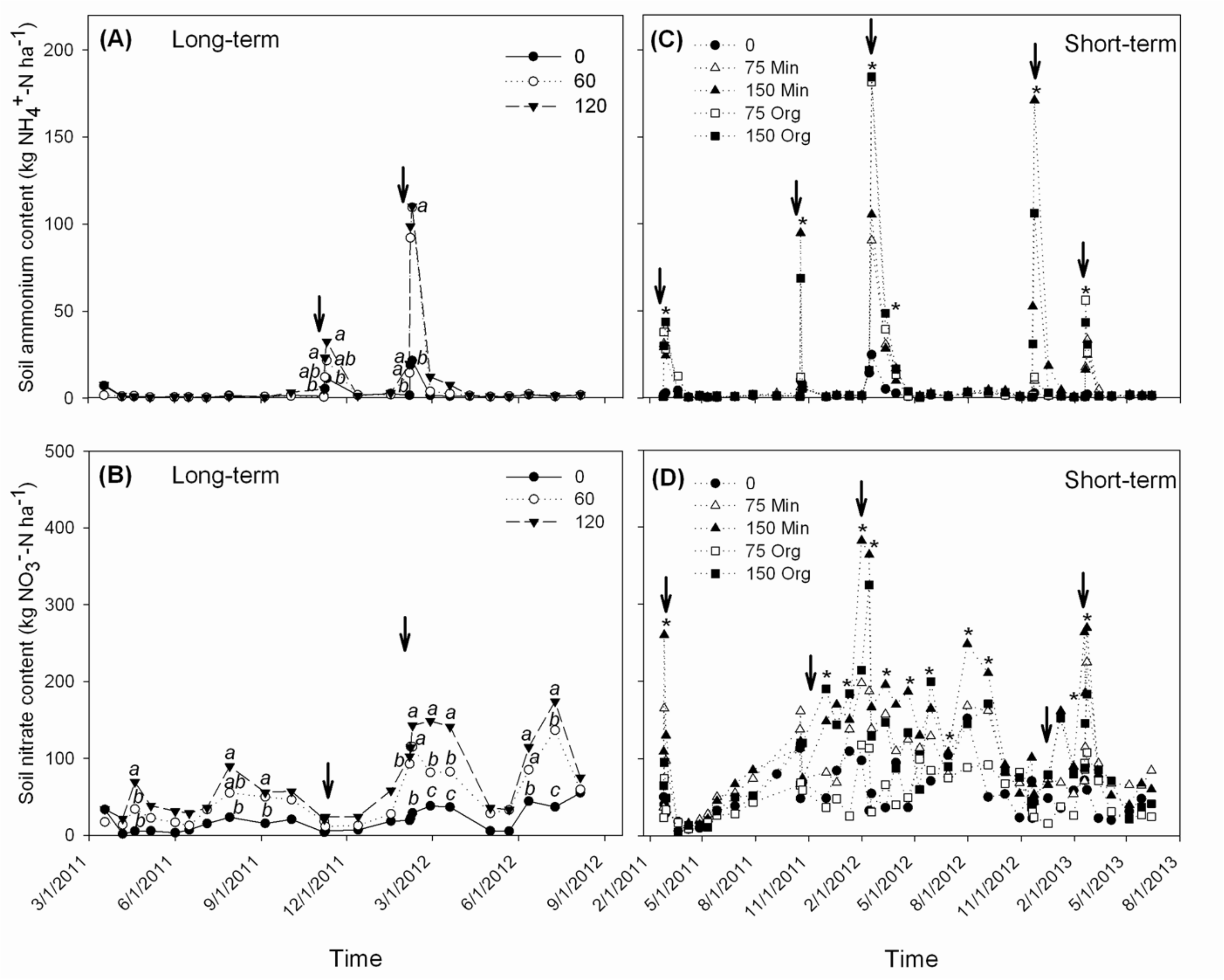
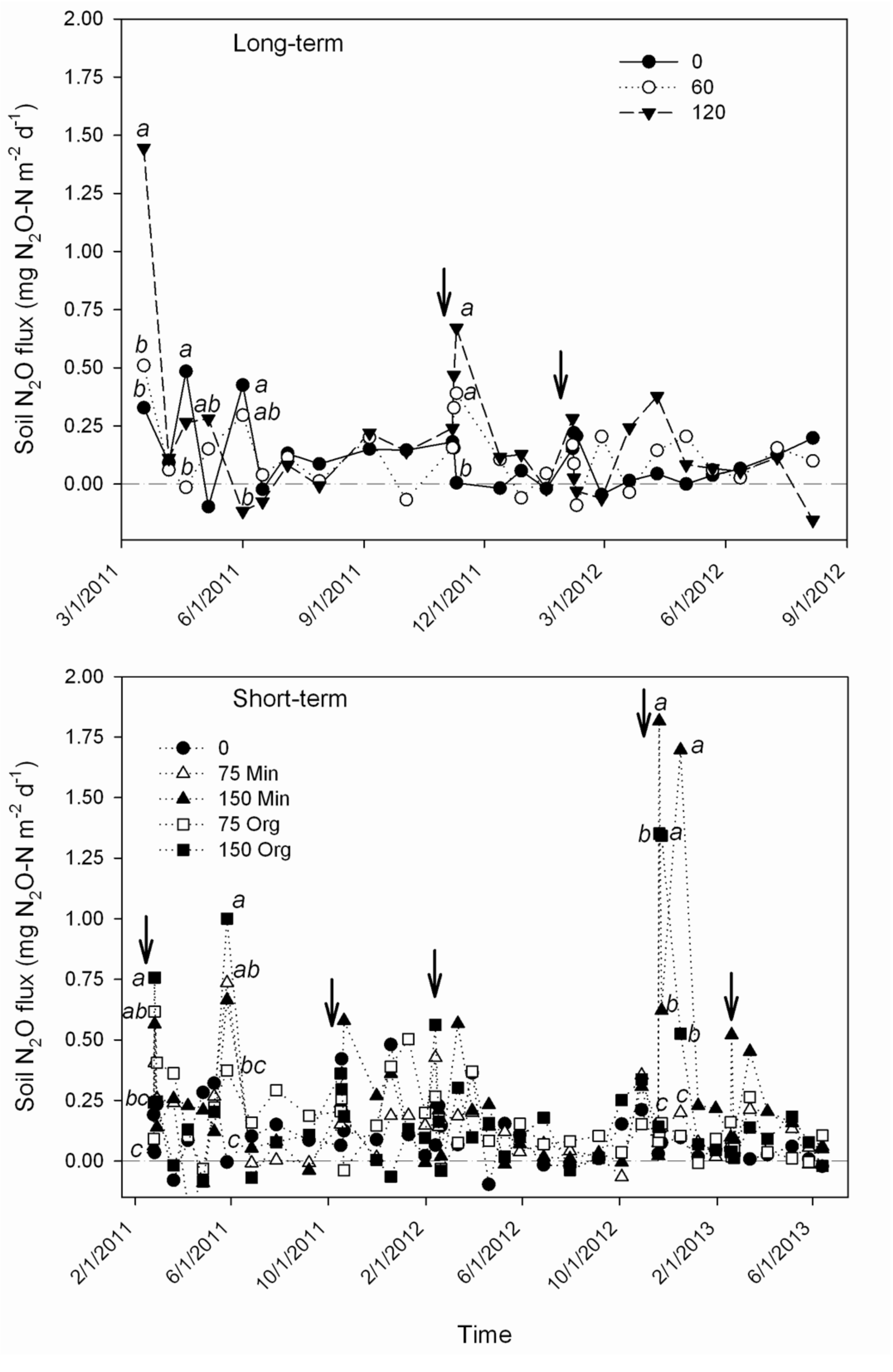

(A) Long-term
(B) Long-term
(C) Short-term
(D) Short-term
0
60
120
75 Min
150 Min
75 Org
150 Org
Soil ammonium content (kg NH_4^+-N ha^{-1})
Soil nitrate content (kg NO_3^--N ha^{-1})
0
50
100
150
200
300
400
500
3/1/2011
6/1/2011
9/1/2011
12/1/2011
3/1/2012
6/1/2012
9/1/2012
2/1/2011
5/1/2011
8/1/2011
11/1/2011
2/1/2012
5/1/2012
8/1/2012
11/1/2012
2/1/2013
5/1/2013
8/1/2013
Time
Time

Fig. 3



Long-term
0
60
120
Soil N$_2$O flux (mg N$_2$O-N m^{-2} d^{-1})
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
a
b
b
a
ab
b
a
ab
b
a
a
b
3/1/2011
6/1/2011
9/1/2011
12/1/2011
3/1/2012
6/1/2012
9/1/2012
Short-term
0
75 Min
150 Min
75 Org
150 Org
a
ab
bc
c
a
ab
bc
c
a
b
a
a
b
b
c
c
2/1/2011
6/1/2011
10/1/2011
2/1/2012
6/1/2012
10/1/2012
2/1/2013
6/1/2013
Time

Fig. 4