

Soil Respiration and N-Mineralization Processes in the Patagonian Steppe Are More Responsive to Nutrient Than to Water Addition

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Abstract

Aims: Soil respiration and N-mineralization are key processes in C and N cycling of terrestrial ecosystems. Both processes are limited by soil temperature, moisture and nutrient content in arid and cold ecosystems, but how nutrient addition interacts with water addition requires further investigation. This study addresses the effects of water and N+P additions on soil respiration and mineralization rates in the Patagonian steppe.

Methods: We measured soil respiration and N-mineralization throughout seasons in control, fertilized, irrigated and irrigated-fertilized plots. We also analyzed root density and soil physico-chemical properties.

Results: The soil CO₂ effluxes in the Patagonian steppe were controlled by soil temperature, soil water content and root density. Increases in water addition had no effects on soil respiration, except when combined with N+P addition. However, soil nutrient enrichment without water addition enhanced soil respiration during the plant growing season. We found a linear positive relationship between root density and soil respiration, without interaction with treatments. N+P addition had negative impacts on N-mineralization, resulting in a strong N-immobilization. However, soil ammonium and nitrate content increased with N+P addition all over the seasons.

Conclusion: Moderate increases in the precipitation through small pulses lead to no long-term response of soil processes in arid and cold Patagonian ecosystems. However, soil CO₂ effluxes are likely to increase with nutrient additions, such as anthropogenic N deposition, and microbial biomass could retain more nutrients in the soil. Therefore, high levels of N enrichment in arid ecosystems may strengthen the positive feedback between C cycle and climate change.

Introduction

Many experimental ecosystem level studies in terrestrial ecosystems have shown significant changes in soil processes such as carbon and nitrogen mineralization (Emmett et al. 2004; Wang et al. 2014b; Abbasi et al. 2020). These processes are extremely sensitive to temperature and precipitation (Fang and Moncrieff 2001; Rustad et al. 2001; Butterbach-Bahl and Gundersen 2011; Hursh et al. 2017) so that climate change could affect key ecosystem functions such as nutrient cycling and carbon storage and cycling. Soil respiration is one of the main processes resulting in C loss to the atmosphere from terrestrial ecosystems, particularly in low leaf area index (LAI) vegetation types such as arid shrublands. In addition, during mineralization carried out by soil microorganisms, organic N is transformed into inorganic through ammonification and nitrification (Butterbach-Bahl and Gundersen 2011), at the same time that CO₂ release due to microbial activity occurs. In recent years it has been recognized that part of the organic matter is also degraded by abiotic factors such as solar radiation (Austin and Vivanco 2006), through which a fraction of CO₂ is released. Both CO₂ efflux and inorganic nitrogen concentration and mineralization rates show a seasonal dynamic and differ between ecosystems (Austin and Sala 2002; Yahdjian et al. 2006; Zhao et al. 2010; Zhang et al. 2011; Reichmann et al. 2013).

Arid and semiarid regions are characterized by low soil respiration (Rustad et al. 2001; Maestre and Cortina 2003) and N mineralization (Mazzarino et al. 1998; Bertiller et al. 2006) rates compared to other ecosystems around the world (Rustad et al. 2001). The arid and semiarid regions are characterized by discrete and unpredictable rainfall events that very rarely permeate to deep soil layers (Sala et al. 1989; Paruelo and Sala 1995). Such rain pulses have crucial impacts on soil C mineralization producing pulses of CO₂ emission into the atmosphere (Kelliher et al. 2004; Jarvis et al. 2007; Silletta et al. 2019). In the same way, microbial decomposition of organic matter and N mineralization is limited to short periods of time when organic matter and soil moisture are higher and soil temperature conditions are mild (Celaya-Michel and Castellanos-Villegas 2011; Dijkstra et al. 2012). Variation in mean precipitation can change plant biomass and allocation patterns and thus affect coupled plant-soil microbial processes. A decrease in soil moisture due to drought could decrease soil respiration and N mineralization (Wang et al. 2014b), while an increase in soil moisture could have the opposite effect (Kelliher et al. 2004; Rey et al. 2016). Moderate increases in soil moisture can enhance root growth and increase root exudations with enzymatic properties that can accelerate the organic matter degradation and microbial activity (Canarini et al. 2019). However, in some water addition experiments there was no response of mineralization rates (Fisher and Whitford 1995), and an increase in soil inorganic N has even been observed under dry conditions in arid ecosystems (Reynolds et al., 1999; Yahdjian et al., 2006).

It is also well known that soil nutrient availability drives many ecosystem processes, such as C mineralization and N transformations (mineralization and immobilization) which govern N availability (Fisk and Fahey 2001; Schaeffer et al. 2003; Lu et al. 2011). Given the low soil N content, water-limited ecosystems can be sensitive and respond to soil nutrient amendments. Although several experiments have been conducted, the effects of N addition on soil respiration are still debated. Some studies indicate that N addition decreases soil CO₂ efflux (Hagedorn et al. 2001; Burton et al. 2004; Janssens et al. 2010), through a decrease in heterotrophic respiration (Treseder 2008; Zhou et al. 2014). However, other studies show that N addition could increase or have no effect on soil CO₂ efflux depending on the responses of the autotrophic and heterotrophic components (Zhou et al. 2014; Zeng et al. 2018). Higher soil nutrient availability can stimulate carbon allocation to roots and thus increase the autotrophic respiration (Zeng et al. 2018; Wei et al. 2020b). On the other hand, higher substrate supply for microbial activity derived from increase in root detrital production and exudation of labile C under nutrient enrichment can favor the heterotrophic respiration (Henry et al. 2005; Ge et al. 2015). In addition, fertilization can increase the aboveground biomass (LeBauer and Treseder 2008) and the nutrient foliar uptake and produce litter with lower C:N ratio (Vourlitis et al. 2009; Lü et al. 2012), promoting litter decomposition and microbial CO₂ release. Some studies reported soil N enrichment promotes microbial mineralization (Contosta et al. 2011; Li et al. 2016), while other studies found negatives or no effects (Fisk and Fahey 2001; Ren et al. 2016; Zhang et al. 2018b; Wang et al. 2019). The effects depend on the applied dose (Li et al. 2016) and duration of experimental N enrichment. Long-term N addition leads to soil acidification, inhibiting soil microbial activity and structural diversity, thereby reducing soil N transformation. Other factors can further compromise N addition effects on C and N mineralization. For example, ecosystems may change towards P limitation as a consequence of improved N availability, limiting the N effect. In some semiarid

ecosystems, P addition was required besides N addition to observe responses of soil respiration (Zhou et al. 2020).

The Patagonian steppe is a cold and arid-semiarid region where water (Bucci et al. 2009; Pereyra et al. 2017) and N and P availability are very scarce (Austin et al. 2004; Bertiller et al. 2006; Gherardi et al. 2013). In this ecosystem, the growing season occurs mainly in spring, when temperatures are increasing and soil water availability is decreasing, so that there is an asynchronism in the key environmental factors which control vegetation growth and soil processes (Austin et al. 2004; Ladwig et al. 2012; Collins et al. 2014). In general, arid ecosystems have received less attention in the study of soil ecological processes than other ecosystems (Xu and Shang 2016). Although there are some studies on the soil respiration (Peri et al. 2015; Silletta et al. 2019) and on soil N-mineralization for some areas of Patagonia (Austin et al. 2004; Yahdjian et al. 2006; Yahdjian and Sala 2008), the effects of the water and nutrient addition and their interaction on these soil processes have not been evaluated together. The main objective of this study was to quantify the effects of the experimental increase in water availability and soil N + P and its interaction on soil respiration and soil N-mineralization throughout the seasons. If an increase in soil moisture or nutrients stimulate the growth and metabolic activity of roots and microbial community, then increased soil respiration and soil N-mineralization are expected. In addition we expected that water and nutrient addition in combination generates synergic effects on these soil processes. A field manipulative experiment was designed in which physico-chemical properties of the soil, root density, soil respiration, ammonification and nitrification were evaluated in control, irrigated, fertilized and irrigated-fertilized plots. Our study can contribute to an improved understanding of the effects of global changes on Patagonian arid ecosystems.

Materials And Methods

Study site

The study was realized at the Rio Mayo Experimental Station of the Instituto Nacional de Tecnología Agropecuaria (INTA, 45°24'11"S; 70°17'37"W), located at the southwest of Chubut Province, Argentina. The site is characterized by a shrub-gramineous steppe from the southern of the Western District of the Patagonian Province (Oyarzabal et al. 2018). Soils are of coarse texture (sandy) with a cemented-calcareous layer at a depth of about 45 cm and have very low organic matter content (0.40%; Paruelo et al., 1988). Mean annual precipitation in the study site is close to 150 mm, and occurs as small events (88% are lower to 5 mm, Cavallaro et al. (2020)). Mean monthly temperature varies from 1°C in July to 15°C in January.

The Patagonian steppe is characterized by vegetation with low species diversity and low plant density, with a plant cover of between 30 and 40% (Pereyra et al. 2017). The vegetation is distributed in patches composed of shrubs and grasses on a bare soil matrix (Soriano et al. 1994). The dominant shrub species are *Azorella prolifera* (Cav.) G.M. Plunkett & A.N. Nicolas (ex *Mulinum spinosum*), *Adesmia volckmannii* Phil. and *Senecio filaginoides* DC. The dominant grass species are *Pappostipa speciosa* (Trin. & Rupr.)

Romasch., *Pappostipa humilis* (Cav.) Romasch. and *Poa ligularis* Nees ex Steud. While shrubs have dimorphic root systems with a taproot and lateral roots (Bucci et al., 2009), grasses have superficial fibrous root systems that are horizontally distributed in the upper 20 cm of the soil profile (Soriano et al. 1987).

Experimental design

Until 2013, the livestock management practice in the study site consisted in a low stocking rate of 0.14 sheep per hectare only from May to October. During the rest of the year the animals were moved to sites with abundant forage. A 4 ha enclosure was delimited in May 2013, and fertilization and irrigation treatments were established. The experimental design consisted of 20 plots of 625 m² each, separated from each other by 15 m, which were randomly assigned to one of the four treatments (control, fertilization (F), irrigation (I) and fertilization-irrigation (I + F)). The plots with the irrigation treatment were equipped with a semi-automatic sprinkler irrigation system. Irrigation water was extracted from a local well of 6 m depth and stored in 5 containers with a total capacity of 13000l. Due to the proximity to the Andean mountains, electric conductivity of groundwater, determined using a conductivity meter (HI 98311, Hanna instruments, Woonsocket, USA) was low (0.16 dS/m), similar to other wells in the research area. The annual precipitation in irrigation plots was increased by approximately 20–25%, and in each irrigation event ~ 5 mm of water were applied, being 6–8 irrigation events per year. The frequency of irrigation events depended on weather conditions (no wind and no natural rain events close to the date of irrigation treatments). Irrigation was carried out during spring and summer, and was canceled during fall and winter, which is the wet period and also to avoid damage to the irrigation system due to freezing of water near the rooting layers. Nutrient addition was done twice a year (once in autumn and once in early spring) applying urea and diammonium phosphate in an amount of 100 kg/ha/year of nitrogen and 75 kg/ha/year of phosphorus, since N and P are the two most limiting nutrients. Another reason for the addition of both nutrients is that nitrogen deposition could further reduce the availability of phosphorus in the soil (Zhang et al. 2013), but the addition of phosphorus could mitigate this limitation, as has been reported for other steppes (Huang et al. 2018).

Soil physico-chemical properties

In spring, 6 cores from the upper soil layer (5 cm diameter, 5 cm depth) were obtained randomly (3 cores close to vegetation and 3 in bare soil) and mixed well to produce one composite sample (n = 3, three of the five plots of each treatment). All soil samples were analyzed for organic matter, total nitrogen, available phosphorus, pH and electrical conductivity. Soil organic matter, total nitrogen and available phosphorus were analyzed in the Soil Laboratory (INTA Chubut, Argentina). Soil organic matter was determined using the wet oxidation method (Walkley and Black 1934). Soil total nitrogen was determined with the Kjeldahl method (Bremner 1996). Available phosphorus was measured using the Olsen method (Olsen et al. 1954). Soil pH was determined in a solution 1:2.5 of distilled and deionized H₂O. Electrical conductivity was determined in a saturated extract and corrected for temperature, and values were reported in dS m⁻¹ at 25°C.

Soil respiration, temperature and water content

Soil respiration was determined in all treatments, twice per season throughout a year. In each plot, one measurement was made near vegetation and the other on bare soil (at least 50 cm away from vegetation), and then the measures were recalculated for the percentage of coverage of each type of patches and then were added together. A portable closed chamber (6400-09, LI-COR) connected to a gas exchange system (LI-6400, LI-COR) was used to measure soil respiration. Soil PVC collars (10 cm in diameter and 4.4 cm in height) were inserted into the soil at 2.2 cm depth one day before each measurement date to allow stabilization of the CO₂ efflux before the measurements. At each measurement date new collars were installed in other sites of each plot. Each measurement took between 5 to 20 minutes, depending on the time of year (in winter the measurements took longer because the rates were relatively low), and included three averaged consecutive cycles. To avoid the effects of strong diurnal fluctuations in air temperature on soil CO₂ efflux, measurements were made between 11:00 and 15:00 h on consecutive days with similar climatic conditions in each study period. In a previous study in this site, Silletta et al. (2019) observed a transient CO₂ release in vegetated patches immediately after a rain pulse of only 3 mm, called "Birch effect". Therefore, soil respiration determinations in this study on irrigation and I + F plots were performed at least five days after irrigation events.

At the time of each soil respiration measurement, soil volumetric water content was measured with ECH2O probes (Decagon Devices, Inc.) at 0–10 cm depth near the soil collars. The probes have an accuracy of 0.03 m³/m³ and a maximum temperature sensitivity of 0.003 m³/m³ per 1°C. The probes were buried on the date previous to the measurements (one probe per collar). The probes were calibrated in the laboratory with soil samples obtained from the study site (Pereyra et al. 2017). Soil temperature was measured at 0–10 cm depth with a soil temperature probe from the LI-6400.

Soil root density

At the end of each soil respiration measurement, soil cores of 10 cm diameter and 10 cm depth inside each soil collar were collected and were transported to the laboratory. Root density was determined in the upper 10 cm of the soil because the highest root density is found in the upper soil layer (0–10 cm) at the study site and decreases exponentially with increasing depth (Pereyra et al. 2017). Fresh soil was carefully separated from the fine roots by sieving the soil through a mesh of 0.5 mm. Fine roots were washed and oven-dried at 70°C until constant weight. Root density was determined as root dry mass per unit volume of soil.

Soil nitrogen dynamics

Soil ammonification and nitrification rates was estimated in situ during each incubation period as the difference between initial and final content of ammonium and nitrate, respectively, in tubes that prevented plant uptake (Raison et al. 1987). PVC tubes of 5 cm diameter and 10 cm height were buried into the soil at 7.5 cm depth, in places close to the vegetation and on bare soil that were later integrated into a single data in three of the five plots of each treatment. The tubes were placed inside the soil in three seasons

(spring, summer and autumn) and they were collected after 1–2 months. At the same time as the placement of the tubes and close to each tube, a soil sample of 5 cm in diameter and 5 cm depth was extracted for the determination of the initial inorganic N content. The collected soil of each tube was transferred into a plastic bag and then transported to the laboratory. Fresh soil samples were sieved with a 0.5 mm mesh, and once frozen they were sent to the Soil Laboratory (INTA Balcarce, Argentina) to analyze the inorganic nitrogen ($\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$) by a distillation method (Bremner and Keeney 1965). The ammonification and nitrification rates were determined from the difference between the initial and final content of ammonium and nitrate in the soil, respectively, during each incubation period, divided by the number of days. Soil inorganic nitrogen and mineralization rates data were converted to aerial basis using bulk density measurements. The $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$ ratio of the soil was calculated for each sample with the initial content values in each of the three seasons.

Statistical analyses

Statistical analyses were performed using the software R version 4.0.1 (R Development Core Team, 2021). We used linear models (LMs) with F-test to evaluate the effect of treatment on soil physico-chemical properties. We used linear mixed effects models (LMEs) with F-test to evaluate the effect of the interaction between season and treatment on soil water content, root density and soil respiration, with month as random effect. A multiple regression model was tested, using LME, to evaluate the effect of the interaction between soil temperature, soil water content and root density on soil respiration, with treatment as a covariate and month as a random effect. For this analysis, continuous variables were centered and scaled, and the quadratic factor of soil temperature and soil water content were included in the model. Linear models with F-test were used to evaluate the effect of the interaction between treatment and season on initial $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ content, $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$ ratio and ammonification and nitrification rates. Linear regressions were tested between the initial $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ content with the ammonification and nitrification rates, respectively, using the previously estimated means per season and treatment. The LMEs were carried out using the function "lme" of the R package "nlme" version 3.1–148 (Pinheiro et al. 2020). The Cox and Snell's R^2 was calculated with the function "nagelkerke" of the R package "rcompanion" version 2.3.25 (Mangiafico 2020). When necessary, models were adjusted using variance models, and the selection of the best model was based on Akaike's information criterion (AIC) (Burnham and Anderson 2002). The simplification of the fixed effects of all models to reach adequate minimum model was carried out by hypothesis test (F-test). Tukey's post-hoc analysis was used for multiple comparisons in all models when the F-test was significant, using the function "glht" of the R package "multcomp" version 1.4–13 (Hothorn et al. 2008).

Results

Lower pH and higher electrical conductivity were observed in fertilized and irrigated-fertilized plots compared to control plots ($p < 0.05$ in all comparisons) (Table 1). Organic matter and total nitrogen did

not show significant differences among treatments (Table 1), but available phosphorus was higher in fertilized ($p = 0.01$) and irrigated-fertilized plots ($p = 0.008$) than in control plots (Table 1).

Maximum values of soil respiration were measured during the vegetation growing season (spring) in all treatments (Fig. 1a). Soil respiration was significantly higher in fertilized ($p = 0.007$) and I + F ($p = 0.01$) plots respect to control in spring (treatment*season: $F_{(9,134)} = 4.36$, $p < 0.001$). Unlike soil respiration, soil volumetric water content (Fig. 1a) was similar between treatments ($F_{(3,143)} = 2.51$, $p = 0.06$), and was higher in winter ($p < 0.001$) compared to summer and autumn, with intermediate values in spring ($F_{(3,4)} = 7.03$, $p = 0.045$). Root density, measured at the same site when respiration was determined, varied significantly between treatments ($F_{(3,150)} = 5.79$, $p < 0.001$), being root density significantly higher in fertilized ($p = 0.008$) and I + F plots ($p < 0.001$), and marginally higher in irrigated plots ($p = 0.078$), compared to control plots (Fig. 1c).

A multiple regression related soil respiration with soil temperature ("temp" in the model), water content ("water" in the model) and root density ("root" in the model). The final simplified model was: respiration \sim temp*water*treatment + temp² + root ($F_{(3,129)} = 7.22$, $p < 0.001$; $F_{(1,129)} = 13.4$, $p < 0.001$ and $F_{(1,129)} = 5.63$, $p = 0.02$, respectively). The 3D plots in Fig. 2 show that the highest values of soil respiration were reached with high values of soil water content combined with intermediate values of soil temperature in F and I + F treatments. In contrast, in control and I treatment the highest values of soil respiration were achieved with the combination of low soil water content values and intermediate to high soil temperature values (Fig. 2). Root density had a linear and positive effect on soil respiration, without interaction with treatment, but with a higher intercept in the F and I + F treatments than in control (Fig. 2, right panel).

Soil mean ammonium-N content ($\text{NH}_4\text{-N}$) at 0–5 cm depth varied between 0.3 and 10 g m^{-2} , being higher in fertilized ($p < 0.001$) and irrigated-fertilized plots ($p < 0.001$) than in control in all seasons (treatment*season: $F_{(6, 24)} = 2.71$, $p = 0.037$; Fig. 3a). Also, $\text{NH}_4\text{-N}$ was higher in summer and autumn than in spring in the I + F treatment. Ammonification rates were significantly lower and negative in F treatment ($p < 0.001$) than in control in all seasons ($F_{(3,32)} = 5.71$, $p = 0.003$; Fig. 3b). In irrigated-fertilized plots, ammonification rates were lower than in control, but it was not significant. In control and irrigated plots, ammonification rates tended to be close to zero.

Soil mean nitrate-N content ($\text{NO}_3\text{-N}$) at 0–5 cm depth varied between 0.2 and 2 g m^{-2} , with significant interaction between treatment and season ($F_{(6, 24)} = 3$, $p = 0.025$). $\text{NO}_3\text{-N}$ was significant higher in F and I + F treatments compared to control plots in spring and summer ($p < 0.01$), and marginally higher in I + F treatment ($p = 0.065$) in autumn (Fig. 3C). Also, $\text{NO}_3\text{-N}$ was higher in spring than in autumn for F and I + F treatments, with intermediate values in summer, without significant differences between seasons for control and I treatment. Nitrification rates were only significantly higher in irrigated plots ($p < 0.001$) compared to control in spring (treatment*season: $F_{(6, 24)} = 2.74$, $p = 0.036$; Fig. 3d). Therefore, nitrification was higher in spring than in summer ($p < 0.001$) and in autumn ($p = 0.02$) for I treatment.

NH₄-N/ NO₃-N ratio varied between treatments and seasons, with significant interaction between both factors ($F_{(6, 24)} = 2.63$, $p = 0.04$). NH₄-N/ NO₃-N ratio was higher in fertilized and I + F treatments, but only was marginally significant in F ($p = 0.05$) and I + F ($p = 0.05$) in spring and in I + F ($p = 0.09$) in autumn (Fig. 4). Moreover, the ratio in the I treatment was marginally higher in spring than in summer ($p = 0.05$) and in the treatment I + F it was marginally higher in autumn than in spring ($p = 0.06$). A significant linear negative relationship was found between NH₄-N and ammonification rate ($R^2 = 0.98$, $p < 0.0001$; Fig. 5a) and between NO₃-N and nitrification rate ($R^2 = 0.495$, $p = 0.003$; Fig. 5b).

Discussion

Responses of soil respiration to water and N + P addition

Several studies have concluded that soil respiration decreases with N addition (Janssens et al. 2010; Ward et al. 2017; Wei et al. 2020a) or no effects are observed (Illeris et al. 2003; Lee and Jose 2003; Qi et al. 2014). However, in our study, the addition of N + P alone or combined with water had a positive effect on soil respiration at least during the growing season (spring), without a synergistic effect of these two treatments on soil respiration. The discrepancy with other studies may be due to the fact that while N deposition may alleviate soil N limitation, this process is accompanied by a reduction in P availability due to an increase in its demand (Gradowski and Thomas 2006; Cao et al. 2011; Li et al. 2016), thus limiting the activity of both roots and soil bacteria. In our study, both macronutrients were added to avoid the limitation due to the low availability of P in the Patagonian steppe. Our results are consistent with those reported in other ecosystems where both nutrients were added together (Mori et al. 2010; Wei et al. 2020b; Zhang et al. 2021). Not consistent with our hypothesis, increased water supply did not stimulate soil respiration in the Patagonian steppe, as it does in other ecosystems (Chen et al. 2013; Lai et al. 2013; Zhang et al. 2017). The determinations of soil respiration in the present study were performed after five days of soil wetting, to avoid large and transient losses of CO₂ due to the filling the air spaces with water (Huxman et al. 2004; Maier et al. 2010). However, in ecosystems where resources are limited, biological activity would be more adapted to respond immediately to short and unpredictable pulses of water (Huxman et al. 2004; Suttle et al. 2007; Collins et al. 2008). In a previous study in the same area we observed a transient CO₂ release pulse in vegetated patches immediately after a rain pulse of only 3 mm (Silletta et al. 2019). Consequently, the present result on non-significant changes in long-term soil respiration would suggest a rapid reversal of CO₂ efflux to background level after a water pulse. In this way, soil CO₂ effluxes to the atmosphere could be underestimated in both treatments (i.e., water addition and water and N + P addition) since CO₂ pulses immediately after irrigation events were not considered. The addition of water by applying small pulses of 5 mm each from the middle of the growing season to the end of the dry season (October-April) accumulated 20–25 mm more than the historical annual average precipitation at the site. Although during the experimental rain pulses the soil water content at 10 cm responded by increasing its content for a short period (Silletta et al. 2019), the added water was not translated into an increase in water availability throughout the year, as it can be seen from our current results. Borken and Matzner (2009) attributed the lack of response of soil respiration to the water addition

to the stock of plant available water in soil, arguing that soil respiration would not be affected by the addition of water until the stock of water available to plants will change significantly.

The combined addition of N and P can stimulate soil respiration through its autotrophic or heterotrophic component (Wei et al. 2020b). Increase in underground biomass observed with nutrient addition in the Patagonian steppe would suggest that higher autotrophic respiration during spring could contribute to soil CO₂ emission. An increase in underground biomass with N and P addition has been previously reported in other ecosystems (Li et al. 2015; Huang et al. 2018). It should be noted that we only evaluated the effects of the treatments on root biomass in the first 10 cm depth, but in this ecosystem more than 40% of roots are found in this soil layer and less than 20% at 50 cm depth (Pereyra et al. 2017). Moreover, the fine roots located in the upper part of the soil profile have higher rates of respiration than larger roots or the few roots located deeper in the soil (Pregitzer et al. 1998). On the other hand, and although the experimental design does not allow us to conclude the reason for the observed increases in CO₂ efflux, it is probably that microbial respiration could also be stimulated with N + P. Shrub and grass leaves in these experimental plots with nutrient addition have a higher N content and a higher total aboveground productivity (Carbonell-Silletta, unpublished work). It may suggest higher litter input and quality which could accelerate decomposition rates and can lead to higher microbial respiration. Furthermore, the higher intercept value in the linear and positive relationship between soil respiration and root biomass in F and I + F plots compared to C and I plots would indicate a higher root metabolic activity per mass unit. We also speculate that under nutrient addition, microbial activity could be stimulated by root exudation of enzymes that accelerate the degradation of organic matter and thus release carbon labile (Ataka et al. 2020). In addition, Song et al. (2011) found a positive relationship between N-immobilization and soil CO₂ efflux with N addition, which reinforces the idea of higher heterotrophic respiration with nutrient addition in our study, where we found a strong N-immobilization.

The lack of response of soil respiration to the addition of N + P from summer to winter can be a consequence of the reduction of the biological activity due to high or low temperatures, respectively, which also coincide with the periods with the lowest and highest soil water content respectively. In the present study it was possible to determine the combined effect of both factors (i.e., soil temperature and water content) on soil respiration. Unlike other studies in which a linear or exponential relationship was found between soil temperature and soil respiration (Hunt et al. 2002; Hursh et al. 2017; Rubio and Detto 2017), we found a quadratic effect of temperature on soil respiration, generally resulting in higher soil respiration values with intermediate temperature values, which occur in the growing season. Similar to studies in other ecosystems (Chang et al. 2014; Wang et al. 2014a), our results support the idea that very low values of soil temperature and moisture restrict soil respiration and this behavior was independent of treatment. However, the pattern was different in the F and I + F treatments with respect to the control with medium to high soil temperature and water content values. In control and the I treatment, the highest values of soil respiration was achieved with intermediate values of soil temperature and moisture, which occurs in spring and coincides with the highest biological activity of plants. However, the highest estimated values of soil respiration in the treatments with N + P addition were obtained with the highest

values of soil temperature combined with the highest values of soil water content. In this steppe there is asynchrony of temperature and soil moisture favorable for the growth of plants, so that the combination of high temperatures with high soil moisture almost never occurs (Paruelo et al. 1998). But, according to our results, it is expected that with the soil nutrient amendment the soil respiration will increase under such conditions, probably due to a greater root density and metabolic activity of the roots and soil microorganisms.

Soil nitrogen dynamics

Compared to other ecosystems, soils of the Patagonian steppe have higher percentage of their mineral nitrogen in form of nitrate (Austin and Sala 2002). The soil nitrogen addition increased N content in the form of ammonium and nitrate. Studies carried out in the same fertilized plots indicates that the two dominant functional groups in the Patagonian steppe (grasses and shrubs) uptakes more N from soil and produce leaves with higher N content than in control plots (Carbonell-Silletta, unpublished work). In this ecosystem, shrubs preferably absorb nitrate rather than ammonium, while ammonium uptake is similar for grasses and shrubs (Sala et al. 2012; Gherardi et al. 2013). The increase in inorganic N content was also accomplished by a substantial increase in the the $\text{NH}_4\text{-N}$ to $\text{NO}_3\text{-N}$ ratio with nutrient soil addition, suggesting higher consumption of nitrate by plants. Another plausible explanation for this change in the ammonium-nitrate ratio is that nitrate is relatively more mobile in the soil solution, being able to leach with water percolation (Yahdjian et al. 2006; Yahdjian and Sala 2010). Soil ammonium and nitrate usually vary seasonally in the Patagonian steppe as a consequence of the differential use by plants and soil microorganisms (Austin and Sala 2002; Yahdjian et al. 2006). However, we only found differences in the N availability between seasons in the treatments with addition of N + P. While the ammonium content was higher in summer and autumn in the I + F treatment, the nitrate content was higher in spring and summer in the F and I + F treatments. The seasonal nitrate behavior in fertilized plots could be explained by high plant demand during the growing season. Some studies in other ecosystems have found increases in soil NH_4 and NO_3 immediately after fertilization, but decrease at the beginning of the rainy season (Kozovits et al. 2007), or soil nitrate decrease after irrigation and fertilization by leaching (Choi et al. 2005).

Nitrogen mineralization in grasslands on a global scale is largely explained by microbial biomass, in addition to the temperature of the wettest quarter, clay content and bulk density (Risch et al. 2019). Contrary to our hypothesis, the negative relationship between inorganic N content and N-mineralization rates found in our study indicates that high N addition could inhibit N transformation, possibly by inhibiting microbial activity (Butterbach-Bahl and Gundersen 2011). Similar stimulation of NH_4^+ and NO_3^- immobilization with increased substrate was observed by Booth et al. (2005) and Song et al. (2021) in a global synthesis. However, Austin and Vivanco (2006) found for the Patagonian steppe that alone N addition has no effect on potential N mineralization, but an increase is observed with the addition of labile C and N. Other studies have also indicated that the addition of nitrogen alone or with water has little or no effects on microbial community in arid ecosystems and it increases when combined with the addition of labile carbon (Austin and Vivanco 2006). The N addition in this study induced significant negative

amination and nitrification rates (i.e.; nitrogen immobilization). In general terms, the balance between N mineralization and immobilization depends on the relative C and N availability of the substrate and the metabolic need of the microbial biomass. Despite exogenous soil nutrient addition, this result implies that more N is needed to supply the microbial demand (Schimel 1986; Micks et al. 2004; Song et al. 2011). If fertilization supplied with more labile C through leaf and root turnover for the microbial growth and activity, then consumption of labile C could result in an increase of N microbial demand and immobilization of available N. Net ammonium and nitrate release only occurs after N demand by microbial biomass, which has lower C:N ratios, was satisfied (Berg and McClaugherty 2003; Song et al. 2011). A compilation of studies about NH_4^+ and NO_3^- (Song et al. 2021) indicates that under high N dose ($> 50 \text{ kg m}^{-2} \text{ yr}^{-1}$) N immobilization increases linearly with N addition rate. When N becomes abundant, microbial communities prefer to utilize mineral N more than organic N (Schimel and Bennett 2004). On the other hand, in N-enriched soils, N immobilization plays an important role in limiting N loss and root intoxication (Zhou et al. 2017; Zhang et al. 2018a).

The addition of N generally leads to soil acidification (Högberg et al. 2006; Vourlitis et al. 2009; Fang et al. 2012), as observed in this study. The global pH decreases by adding N is 0.26 for terrestrial ecosystems (Tian and Niu 2015), however the change observed in the present study was higher (0.75 and 0.62 in irrigated and irrigated-fertilized plots respectively). These values are more consistent with the results observed in grasslands of northern China (0.63), mainly induced by N deposition (Yang et al. 2012). Ecosystems vary in their sensitivity to acidification and this is dependent on the initial pH, the decrease being greater the more basic the initial pH is (Tian and Niu 2015). The soils of the Patagonian steppe have a high calcium carbonate content (Paruelo et al. 1988; del Valle 1998) indicating that they may be very sensitive to acidification by the N addition. The acidification observed in our study could explain, at least in part, the strong inhibition of soil mineralization due to its effect on microbial activity by affecting enzyme functioning (Li et al. 2018; Nannipieri et al. 2018).

The water addition did not significantly modify the content of mineral N and the N-mineralization rates, consistent with the results of studies carried out in another arid ecosystem (Reichmann et al., 2013; Hook and Burke 2000). But this finding contrasts with the results of Yahdjian and Sala (2010) for the Patagonian steppe. However, it should be noted that in this last study the mineralization rate was determined only 5 days after the addition of water, so the increase could represent only pulses of N availability, as previously reported (Epstein et al., 2006). Generally, C and N mineralization after rewetting are coupled (Fierer and Schimel 2002; Muhr et al. 2010), such that the response of N mineralization, similar to soil respiration, was not captured in our study. After microbial growth due to rewetting, an increase in metabolic microbial activity (Austin et al. 2004) and microbial death are observed simultaneously with the depletion of labile soil organic matter, leading to large pulses of N mineralization. Our results indicate that the addition of relatively small water pulses does not lead to long-term responses.

Conclusions

The main control factors on the soil respiration in the Patagonian steppe were soil temperature, water content and root density. However, the experimental water supply did not produce a significant effect on the release of CO₂ and N mineralization over the long term. This emphasizes the need to evaluate these soil processes with higher frequency after soil wetting to avoid underestimations of soil C loss in future studies. In this study the increase in mean precipitation was carried out with small precipitation events (~5mm), thus we cannot rule out that increasing the water pulse size may trigger long-term responses.

On the other hand, the soil respiration increased with the addition of N and P during the growing season. More roots in F and I + F treatments stimulated the loss of soil carbon but there were not synergic effects of nutrient and water. Therefore, we suggest that soil N enrichment in arid ecosystems may strengthen the positive feedback between climatic change and C cycle. However, other studies are required to determine if only N addition without P result in higher CO₂ effluxes from the soil.

Nutrient addition also resulted in changes in soil inorganic N, affecting soil N mineralization rates. Although the soil inorganic N in the form of ammonium and nitrate increased with fertilization, these two forms of N compounds were immobilized by microorganisms. The low mineralization rates found even in the control plots would suggest that soil mineralization processes in the Patagonian steppe is restricted to short periods during the dry season in which rainfall pulses occur. However, how these changes modify the net C and N balance of the ecosystem were not explored, and further studies are necessary to assess the relative responses of various C and N cycles components to nutrients and water addition. While most studies in arid ecosystems have paid more attention to water availability, our results suggest that we must consider the effects of soil nutrients to better predict changes in C fluxes and C net balance in this ecosystem.

Declarations

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Conflicts of interest: The authors declare that they have no conflicts of interest.

Data availability: The datasets generated and/or analyzed during this study are available from https://osf.io/qjyjf/?view_only=8588916d83054b5e91512f7c8a984f1d.

Code availability: The R code generated during this study is available from https://osf.io/qjyjf/?view_only=8588916d83054b5e91512f7c8a984f1d.

Authors' contributions: Fabian Scholz and Sandra Bucci conceived the ideas and designed methodology. Luisina Carbonell-Silletta, Agustin Cavallaro, Daniel Pereyra and Javier Azkenazi collected the data. Luisina Carbonell-Silletta analysed the data. Luisina Carbonell-Silletta, Sandra Bucci and Guillermo

Goldstein wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Tables

Table 1

pH, electrical conductivity (dS m^{-1}), organic matter (%), total nitrogen (%) and available phosphorus (ppm). Each value represents the mean \pm CI ($n = 3$). The statistical value F, the degrees of freedom (df) and the probability value p are shown. Statistically significant differences of a treatment with respect to control are labeled with asterisks (* $p < 0.05$)

	Treatment				Test-F
	C	F	I	I+F	
pH	7.15 (0.20)	6.40 * (0.20)	7.10 (0.20)	6.48 * (0.20)	$F_{(3,8)} = 20.2$ $p = 0.0004$
Electrical conductivity (dS m^{-1})	0.20 (0.11)	0.54 * (0.11)	0.17 (0.10)	0.43 * (0.10)	$F_{(3,8)} = 15.7$ $p = 0.001$
Organic matter (%)	0.75 (0.21)	0.82 (0.21)	0.74 (0.21)	0.84 (0.21)	$F_{(3,8)} = 0.35$ $p = 0.79$
Total N (%)	0.044 (0.016)	0.044 (0.016)	0.041 (0.017)	0.050 (0.017)	$F_{(3,8)} = 0.28$ $p = 0.84$
Available P (ppm)	14.8 (17.4)	54.7 * (17.4)	15.6 (17.4)	59.4 * (17.4)	$F_{(3,8)} = 10.3$ $p = 0.004$

Figures

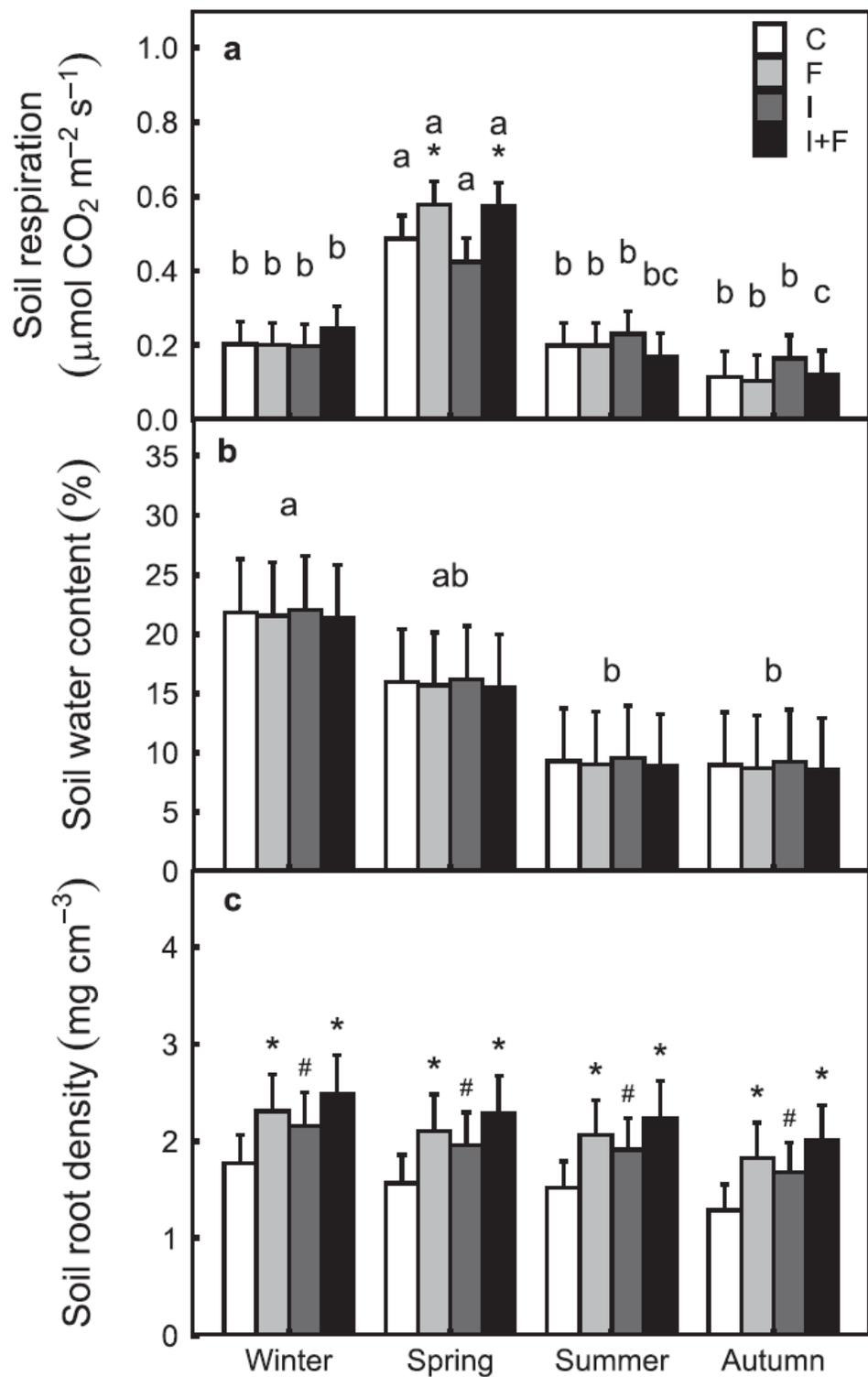


Figure 1

(a) Soil respiration ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), (b) root density at 0-10 cm depth ($\text{mg dry mass cm}^{-3}$), and (c) water content at 0-10 cm depth (%), for four treatments (control (C), fertilization (F), irrigation (I) and irrigation-fertilization (I+F)) in each season. Each bar represents the mean \pm CI (n= 5). Statistically significant differences of a treatment with respect to control for each season and site are indicated as * $p < 0.05$. Marginally significant differences of a treatment with respect to control for each season and site

are indicated as # $p < 0.1$. Different lowercase letters indicate significant differences between seasons for each treatment

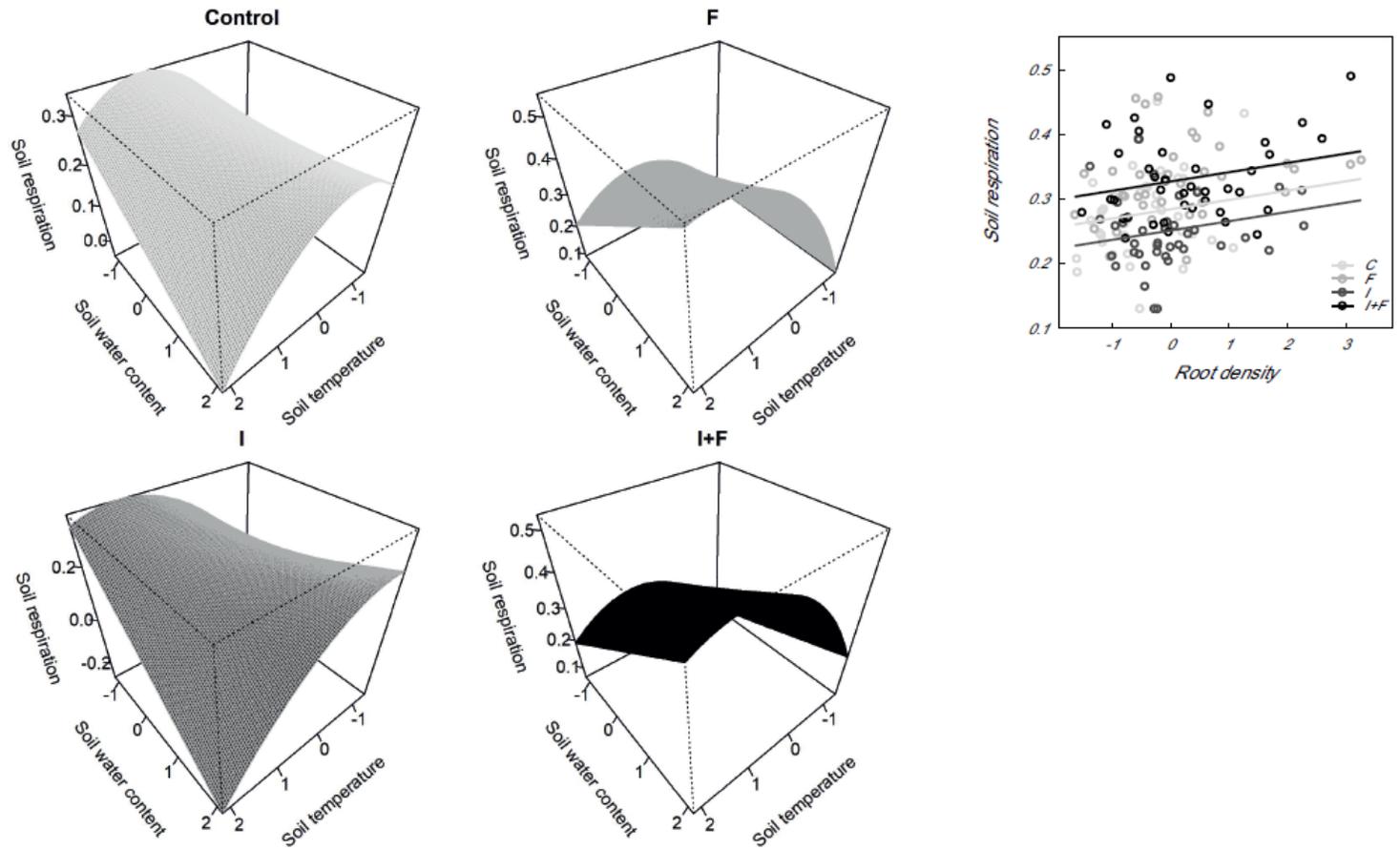


Figure 2

Plots of results of the multiple regression. On the left, 3D plots of soil respiration in relation to soil water content and soil temperature for each treatment: control (C), fertilization (F), irrigation (I) and irrigation-fertilization (I+F). On the right, soil respiration in relation to root density for each treatment. The values were centered and scaled for the analysis

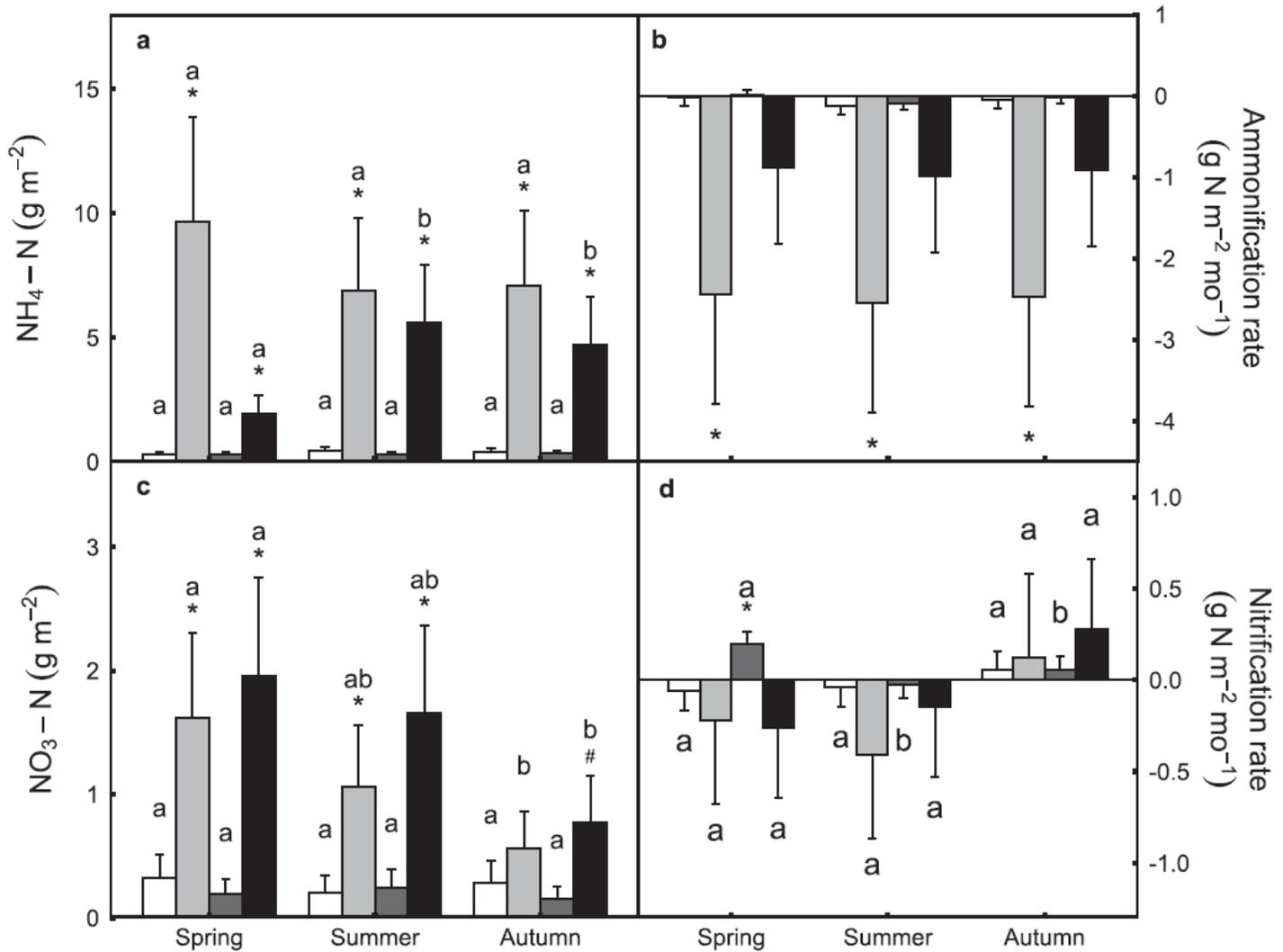


Figure 3

Soil ammonium-N ($\text{g NH}_4\text{-N m}^{-2}$) and nitrate-N ($\text{g NO}_3\text{-N m}^{-2}$) in panels a and c respectively, and ammonification and nitrification rates ($\text{g N m}^{-2} \text{mo}^{-1}$) in panels b and d respectively, in spring, summer and autumn for each treatment: control (C), fertilization (F), irrigation (I) and irrigation-fertilization (I+F). Each bar represents the mean \pm CI (n = 3). Statistically significant differences of a treatment with respect to control for each season and site are indicated as * p < 0.05. Marginally significant differences of a treatment with respect to control for each season and site are indicated as # p < 0.1. Different lowercase letters indicate significant differences between seasons for each treatment

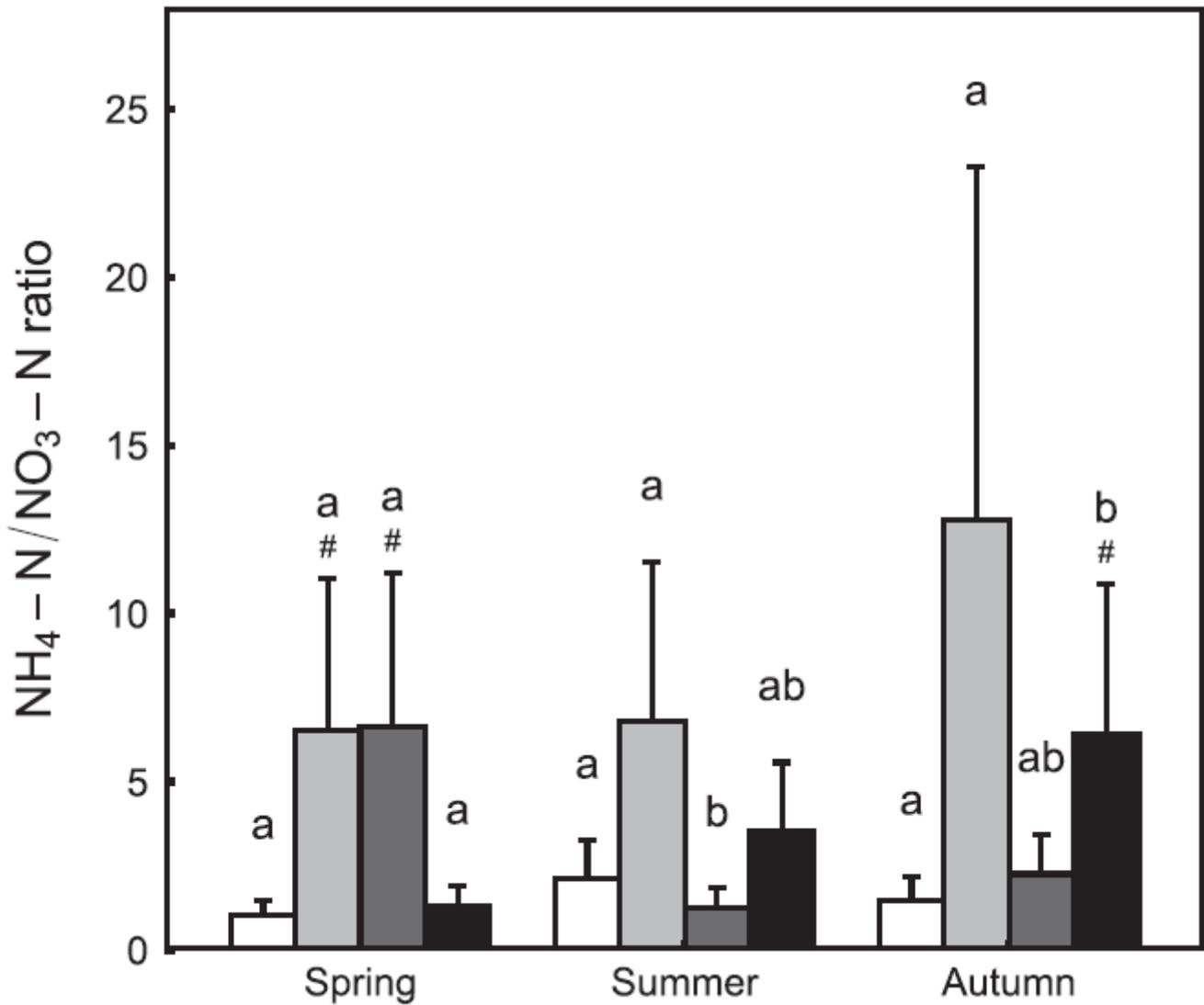


Figure 4

Ammonium (NH₄-N) / nitrate (NO₃-N) ratio for each treatment: control (C), fertilization (F), irrigation (I) and irrigation-fertilization (I + F). The values are the means ± CI (n = 3). Marginally significant differences of a treatment with respect to control for each season and site are indicated as # p < 0.1. Different lowercase letters indicate significant differences between seasons for each treatment

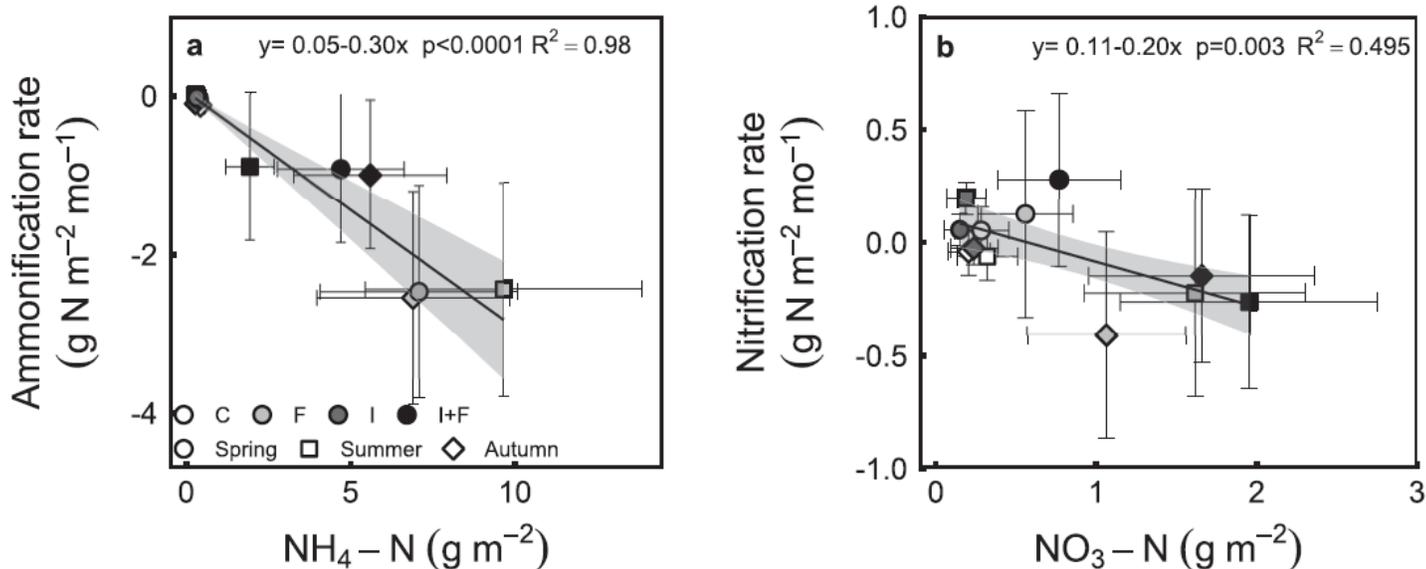


Figure 5

(a) Soil ammonification rate ($\text{g N m}^{-2} \text{ mo}^{-1}$) related to N content in the form of ammonium ($\text{g NH}_4\text{-N m}^{-2}$), and (b) soil nitrification rate ($\text{g N m}^{-2} \text{ mo}^{-1}$) related to N content in the form of nitrate ($\text{g NO}_3\text{-N m}^{-2}$). Each value represents the mean \pm IC ($N = 3$) of each treatment: control (C), fertilization (F), irrigation (I) and irrigation-fertilization (I+F) and season (spring, summer and autumn). The lines are the linear regressions fitted to the data and the gray bands are 95% confidence intervals