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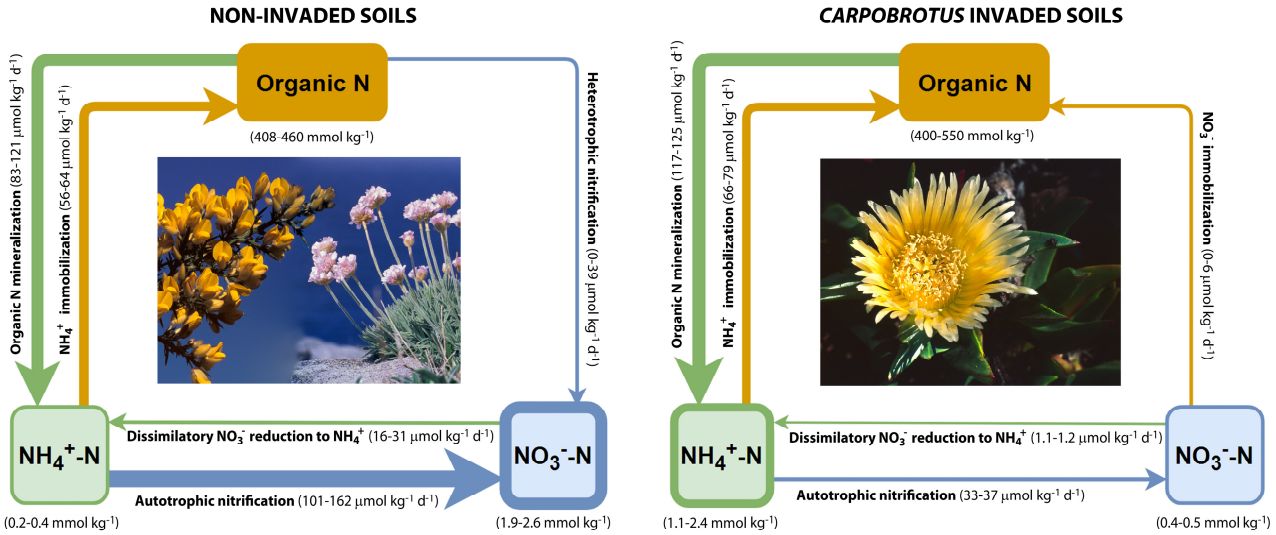
Highlights

- Gross N fluxes in *C. edulis* invaded and uninvaded soils were modelled with *Ntrace.*
- *Carpobrotus edulis* invasion affected gross N fluxes in the 0-5 and 5-10 cm layers.
- Invasion reduced NO_3^- producing and consuming rates and raised NH_4^+ immobilization.
- Invaded soils showed lower net nitrification, N mineralization and N availability.
- D_{NRA} was usually the exclusive/dominant NO_3^- consumption rate in our C-rich soils.



Abstract

The effects of alien plants on whole nutrient cycles have been scarcely studied, despite the increasing evidence on their impact on nutrient pools and fluxes. *Carpobrotus edulis*, a dangerous invasive plant in coastal areas worldwide, is considered an ecosystems engineer which, by changing many soil properties, benefits its own invasion and hampers the restoration of the invaded habitats. To study, for the first time, the 'true' impact of *C. edulis* on the soil N cycle, we used a paired ^{15}N labelling experiment and a *Ntrace* compartment model to estimate the gross N fluxes in the 0-5 cm and 5-10 cm soil layers of non-invaded and *C. edulis* invaded areas of two temperate-humid coastal rocky locations. *Carpobrotus edulis* invasion generally increased NH_4^+ immobilization (I_{NH4}, 1.19-4.48x), presumably due to a lower N availability for the microbiota. The invasion also decreased autotrophic nitrification (O_{NH4}, 0.20-0.79x), either by a direct effect over soil microbiota or by the acidification triggered by *C. edulis*. Unexpectedly, the dissimilatory nitrate reduction (D_{NRA}) was the exclusive NO_3^- consuming process modelled on most of the studied soils, although the incubation was aerobic. Apparently, the high organic C content of these soils induced a higher O_2 consumption and the formation of anaerobic microsites where the D_{NRA} could have taken place. The lower NO_3^- availability of invaded soils could explain their lower D_{NRA} rates (0.04-0.70x) compared to native soils. Both D_{NRA} and O_{NH4} were more affected in the 0-5 cm layer, but the invasion also significantly affected N rates in the 5-10 cm layer. Overall, net nitrification and mineralization generally decreased in the invaded soils. This study shows that the invasion of *C. edulis* alters soil gross and net N fluxes in a 0-10 cm depth through its effects on soil properties and microbiota.

Keywords Alien plants · Autotrophic nitrification · Dissimilatory NO_3 reduction · Heterotrophic nitrification · N mineralization · N immobilization

1. Introduction

Plant invasions can impact the base of all terrestrial ecosystems - the soil - by modifying its physical and chemical characteristics, nutrient cycling and microbiota (Dassonville et al., 2008; Ehrenfeld, 2003; Ehrenfeld and Scott, 2001; Suding et al., 2013; Vanderhoeven et al., 2005; Weidenhamer and Callaway, 2010). Of particular importance are the invaders' effects on the N cycle, as N is the most widespread limiting nutrient in ecosystems (Galloway et al., 2004; Vitousek and Howarth, 1991) and N availability can affect plant community structure. Therefore, changes on soil N pool, N residence time and ecosystem N cycling feedbacks can provide a competitive advantage of invaders over natives, facilitating the invasion (Laungani and Knops, 2009; Theoharides and Dukes, 2007).

Invasive plants can modify the soil N cycle through their impacts on soil microbial communities, litter decomposition and soil properties (Laughlin, 2011; Schaeffer et al., 2012; Wang et al., 2015). These changes can be driven by differences in phenology, leaf traits, plant litter composition, N use, N residence time, interaction with herbivores, symbiosis with native or co-introduced N_2-fixing bacteria, effects on soil microbiota structure and activity, and effects on the microclimate (Castro-Díez et al., 2014; Corbin and D'Antonio, 2004; Knops et al., 2002; Laughlin, 2011; Laungani and Knops, 2009; Lee et al., 2017; Mack and D'Antonio, 2003). The modifications of the N cycle can be influenced by the characteristics of the invaded site and the invasive species, being often stronger in mild climates and islands (Castro-Díez et al., 2014) and when the traits of the invasive plant differ from those of the native flora (Castro-Díez et al., 2014; Lee et al., 2017).

The effects of invasive plants on the soil N cycle are species and context-dependent (Wang et al., 2015), but usually involve increases on the N pools and fluxes (Lee et al., 2017). Generally, a positive plant-soil feedback - higher litter quality of the invader resulting in higher decomposition rate and N availability - is found (Knops et al., 2002; Liao et al., 2008; Stark and Norton, 2015; Wang et al., 2015).

Furthermore, impacts of invasive plants on the soil N cycle can persist after their removal (Elgersma et al., 2011). This is particularly true when invasions involve changes in microbial communities (Elgersma et al., 2011) or in N stock and availability, affecting the recolonization patterns and the restoration of the ecosystem (Corbin and D'Antonio, 2004), and increasing the risk of secondary invasions by ruderal nitrophilous plants (Novoa et al., 2013; Santoro et al., 2012; Santoro et al., 2011). As net N fluxes are the result of several counteracting processes (Murphy et al., 2003), they do not adequately reflect the impacts of invasive plants (Piper et al., 2015). Therefore, studies on the effect of invasive plants on the gross N fluxes are necessary to fully understand how the invasion is disturbing the N cycle. However, these studies are scarce and mostly focussed on annual grasses (Booth et al., 2003; Hawkes et al., 2005; Parker and Schimel, 2010; Piper et al., 2015; Schaeffer et al., 2012; Stark and Norton, 2015), with only a few exceptions for perennial grasses (Thorpe & Callaway, 2011), leguminous plants and trees (Laungani and Knops, 2012).

Carpobrotus edulis (L.) N.E.Br is a perennial mat-forming succulent plant from the Aizoaceae family (Campos et al., 2004). Although it is original from South Africa, it has been introduced worldwide with ornamental, medical and land stabilisation purposes (Campos et al., 2004; Malan and Notten, 2006; Maltez-Mouro et al., 2010). Currently, it is considered a highly invasive plant outside its native range (Global-Invasive-Species-Database, 2016; Vilà et al., 2006); the same is true for its hybrid *Carpobrotus aff. acinaciformis* (L.) L. Bolus.

It has been repeatedly proved that *C. edulis* affects the physicochemical properties (Conser and Connor, 2009; Novoa et al., 2013; Novoa et al., 2014; Vieites-Blanco and González-Prieto, 2017; Vilà et al., 2006) and microbiota (de la Peña et al., 2010) of the invaded soil. Through its effects on soil and other abiotic elements, *C. edulis* favours its own invasion (Conser and Connor, 2009), and the establishment of opportunistic native species as well (Novoa et al., 2013), so it is considered an ecosystem engineer (Conser and Connor, 2009; Molinari et al., 2007). This alien invasive plant can increase (Badalamenti et al., 2016; Santoro et al., 2011) or decrease (Conser and Connor, 2009; Vieites-Blanco and González-Prieto, 2017) soil total N, and increase (Novoa et al., 2014) or decrease (Novoa et al., 2014; Vieites-Blanco and González-Prieto, 2017) soil NH_4^+ and NO_3^- availability. This variability on the magnitude and direction of the impacts of *C. edulis* on the invaded soil suggests a context-dependency of its effects (Vieites-Blanco and González-Prieto, 2017), also seen for other invaders (Vilà et al., 2011). Moreover, its necromass has a higher C/N ratio than native necromass (Badalamenti et al., 2016; Vieites-

Blanco and González-Prieto, 2017). The impact of *C. edulis* on gross N fluxes has not been studied yet, despite having this alien invasive species site-dependent effects over N compounds (Novoa et al., 2013; Novoa et al., 2014), and being still controversial the interpretation of the processes involved in these changes.

Therefore, the aim of the present study was to evaluate, for the first time, the effect of *C. edulis* invasion on gross N rates. We used a paired ^{15}N labelling experiment and the state-of-the-art *Ntrace* compartment model (Müller et al., 2007) to estimate the gross N fluxes in the 0-5 cm and 5-10 cm soil layers (the most affected by alien roots and necromass) of non-invaded and *C. edulis* invaded areas in two temperate-humid rocky areas (NW Spain shoreline).

2. Material and methods

2.1. Site and sampling description

Two representative study sites of the coastal rocky areas invaded by *C. edulis* were selected in NW Spain: Punta Nariga (43º19'13'' N, 8º54'34'' W; recently invaded: 15-20 years) and Sálvora Island (42º27'55'' N, 9º0'49'' W; long-term invasion: ~80 years); both of them with Umbric leptosol soils (IUSS Working Group, 2014) formed over granitoid rocks. Climate is characterised by a mean annual temperature of 14-16 ºC and a mean annual precipitation of 1400-1800 mm (AEMET-IMP, 2011). The distance to the sea was similar in both sites: 70-80 m in Punta Nariga and 65-95 m in Sálvora. The non-invaded areas were dominated by *Ulex europaeus* L., *Erica vagans* L. and *Armeria pubigera* (Desf.) Boiss. in Punta Nariga, and by *Armeria pubigera* in Sálvora.

In September 2015, for each location, the soil 0-5 and 5-10 cm layers were separately sampled in 10 randomly distributed 15x15 cm squares under native vegetation and another 10 under *C. edulis.* Soil subsamples were mixed into a composite sample per site and depth. The soils were sieved (< 2 mm), homogenized and kept at 4 ºC. The main characteristics of soils are reported elsewhere (Vieites-Blanco and González-Prieto, 2017).

2.2. Soil incubation and gross N transformation rates

Before starting the experiment, soils were wetted to slightly below 70% of their water holding capacity with the wetting system described in Gómez-Rey and González-Prieto (2013), which allows easily wetting highly hydrophobic soils. A paired ^{15}N labelling experiment, with $^{15}NH_4NO_3$ and $NH_4{}^{15}NO_3$ as tracers and four incubation times (0.5 h, 1, 3 and 7 days), was conducted. Aliquots equivalent to 30 g of dry soil were placed in a total of 192 centrifuge bottles (250 ml): two invaded soils, two non-invaded soils, two soil depths, two ^{15}N-tracers, four incubation times and three replicates. The aliquot of soil corresponding to each bottle was deposited as 4 successive layers, each of which received 1 mL of ^{15}N-tracer solution added uniformly over the soil surface with an automatic pipette (i.e. 4 mL per bottle), equivalent to an N addition of 1 mg kg^{-1} dry soil with a ^{15}N excess fraction of 49 %. After labelling, soils were incubated at 25 ºC in darkness and, then, an extraction-diffusion method was used for NH_4^+-N and NO_3^--N quantification. Briefly, soils were extracted with 150 mL KCl 2M (1:5 soil:solution ratio), shaken for 1 h and passed through glass microfibre filters (Whatman GF/A, 125 diameter). Ammonium and nitrate were sequentially liberated with two consecutive microdiffusions (55 ºC, 72 h) from 50 mL aliquots placed in 500 mL glass jars, by adding respectively MgO (0.2 g) and MgO (0.2 g) plus Devarda's alloy (0.4 g). Both N forms were trapped as NH_3 into 10 mL of 0.004 M H_2SO_4 in a Teflon bottle suspended in the glass jar. Measurement was made by back titration of the H_2SO_4 excess with 0.004 M NaOH. Three blanks and three standards (NH_4NO_3) were included in each batch to subtract N from reagents and to check for N recovery. After the two titrations (for NH_4^+ and NO_3^-), the resulting $(NH_4)_2SO_4$ solutions were evaporated to dryness at 60 °C in a vacuum oven (Memmert VO400, PM400) for obtaining $(NH_4)_2SO_4$ crystals. To accelerate the drying process, the oven was alternatively under vacuum (15 kPa) and atmospheric pressure. In order to trap possible traces of atmospheric NH_3, the incoming air was passed through a column of activated charcoal. The $(NH_4)_2SO_4$ crystals were packed into tin capsules and analysed for ^{15}N. The soil remaining in the flasks and the filters during the extraction procedure were washed with deionized water until no chlorides were detected (silver nitrate test), oven-dried at 105 ºC, finely ground (< 100 µm) and packed into tin capsules for organic N analyses. The ^{15}N enrichment of NH_4^+-N and NO_3^--N, as well as the organic N content and its ^{15}N enrichment were measured with an elemental analyser (Carlo Erba CNS 1508) coupled on-line with an isotopic ratio mass spectrometer (Finnigan Mat, delta C, Bremen, Germany). An elemental reference material (Soil 3 from Eurovector, Milano, Italy)

and an isotopic standard (IAEA-N1, IAEA-N2, IAEA 305-A, IAEA 305-B and IAEA 311, alternately, from the International Atomic Energy Agency, Vienna, Austria) were included in each set of 10 samples to check the accuracy of the results; if necessary, drift correction was made against internal standards during the run.

2.3. ^{15}N-tracing model

To quantify gross N transformation rates, a *Ntrace* compartment model (set up in Simulink and summoned by the MCMC optimisation routine programmed in MatLab; The Math Works Inc.) with different N pools and possible N transformations was suggested as a start point (Müller et al., 2007; Rütting and Müller, 2007).

No evidences of abiotic NH_4^+ fixation were found and, consequently, only two pools of inorganic N (exchangeable NH_4^+, exchangeable NO_3^-) were considered for the modelling. Following Nelissen et al. (2012), the soil organic nitrogen (SON) was compartmentalized in a microbially easily available fraction (SON_{lab}, 1% of total SON) and a more difficult to mineralize fraction (SON_{rec}, 99% of total SON) as this often helps to better model the mineralization rate of SON. The initial pool sizes and ^{15}N abundance for NH_4^+ and NO_3^- were estimated by extrapolating back to t=0 the measurements from the first two soil extractions (t=0.5 hours and t=1 day) (Müller et al., 2004). Depending on the considered soil, the model was run 7-15 times changing both the number of rates and the combinations of them until the most fitting model to our data was found. In all soils, we checked from the simplest model, with only the most usually considered gross N rates (mineralization, nitrification, NH_4^+ and NO_3^- immobilization), to the most complex *Ntrace* model possible with our data, which would include: a) M_{SONrec}, mineralization of SON_{rec} to NH_4^+; b) I_{NH4rec}, immobilization of NH_4^+ to SON_{rec}; c) M_{SONlab}, mineralization of SON_{lab} to NH_4^+; d) I_{NH4lab}, immobilization of NH_4^+ to SON_{lab}; e) O_{SON}, oxidation of SON to NO_3^- or heterotrophic nitrification [NO_3^- production from SON without mixing into the free soil NH_4^+ pool; see Barraclough and Puri (1995) for assumptions and possible limitations]; f) O_{NH4}, oxidation of NH_4^+ to NO_3^- or autotrophic nitrification; g) I_{NO3}, immobilization of NO_3^- to SON; and h) D_{NRA}, dissimilatory nitrate reduction to ammonium. *Ntrace* is also able to model N losses by denitrification and NH_3 volatilisation, but these rates were discarded taking into account that the recovery of the added ^{15}N showed nil or negligible N losses. All transformations were described by first order kinetics, except M_{SONrec} and O_{SON} which followed zero order kinetics. If the values yielded by the model for a certain rate were close to zero, they did not follow a normal distribution and the inclusion of the rate did not improve the performance of the model, the rate was not retained in the model following the Akaike information criterion (Staelens et al., 2012). According to this, M_{SONrec} was not considered in any of the modelled soils, being the other N rates retained at least in one of the studied soils.

The parameters of the selected transformation rates were estimated using a Markov chain Monte Carlo (MCMC) method by fitting the model values to the measured contents and ^{15}N enrichments of NH_4^+ and NO_3^- (Müller et al., 2007). The optimisation procedure results in a probability density function (PDFs) from which parameter averages and standard deviations were calculated (Müller et al., 2007). For transformations following first order kinetics, average rates were calculated by integrating gross N rates over the experimental period divided by the total time (Rütting and Müller, 2007). Standard errors of means were calculated based on autocorrelation as described in Harmon and Challenor (1997). Due to the high number of iterations used in the model, the usual statistical tests cannot be used (Rütting et al., 2010). Alternatively, statistical significance in differences between treatments was tested by an overlap of the 85% confidence intervals (CI) (Payton et al., 2000; Rütting et al., 2010).

Net N transformations were calculated from the obtained gross rates: net ammonification [$M_{SONlab} - (I_{NH4lab} + I_{NH4rec})$], net nitrification [$(O_{NH4} + O_{SON}) - (D_{NRA} + I_{NO3})$] and net N mineralization (sum of both net rates).

3. Results

3.1. N pools

The initial NH_4^+-N pool was usually higher in the *C. edulis* invaded soils than in those under native vegetation, differences being wider for the 0-5 cm layer (Figs. 1-4). While the amount of NH_4^+-N decreased during the incubation in the uninvaded soils (irrespectively of soil depth), it showed contrasting tendencies in the invaded soils: increase in the surface layer and decrease in the sub-surface one (Figs. 1-4). Conversely, the initial NO_3^--N pool was higher in soils under native vegetation than in the *C. edulis* invaded

soils and its size always increased during the incubation (Figs. 1-4).

3.2. Gross N fluxes

In general, there was a good fit between the measured and the *Ntrace* modelled data of the size and ^{15}N abundance of the NH_4^+-N and NO_3^--N pools (see Figs. 1-4).

Irrespectively of vegetation cover and soil depth, no satisfactory adjustments were obtained for models with two organic N pools (SON_{lab} and SON_{rec}: 1% and 99% of total SON, respectively) and two mineralization rates (M_{SONlab} and M_{SONrec}, with first and zero order kinetic, respectively). In all cases, the best fittings were obtained when only the mineralization of the labile fraction (M_{SONlab}) was considered, because the values yielded by *Ntrace* for M_{SONrec} were not normally distributed and its inclusion never improved model fitting; consequently, this rate was not included in the finally selected models. Except in the Nariga soil under native vegetation, the M_{SONlab} was significantly higher in the topsoil than in the 5-10 cm layer (Fig. 5a). The effect of *C. edulis* invasion in the M_{SONlab} rate was site- and depth-dependent, not showing a clear trend.

The gross immobilization of NH_4^+ to recalcitrant N (I_{NH4rec}) was only modelled for surface soils under native vegetation, accounting for more than half of the total NH_4^+ immobilization in these soils (Fig. 5b). As for M_{SONlab}, except in the Nariga soil under native vegetation, the gross immobilization of NH_4^+ to labile N (I_{NH4lab}) was significantly higher in the topsoil than in the 5-10 cm layer (Fig. 5a,b). *Carpobrotus edulis* invasion increased the I_{NH4lab} rate in surface soils (2.81 to 3.07x; 1.19x when considering I_{NH4rec}+I_{NH4lab}) and it had contrasting effects within sites in the 5-10 cm layer (0.79x for Nariga, 4.48x for Sálvora).

Regarding NO_3^--N production processes, gross oxidation of recalcitrant N (O_{Nrec}, i.e. heterotrophic nitrification) was only modelled in the uninvaded surface soil of Nariga site (Fig. 5c), the inclusion of this rate in the model being necessary because it improves by 23% the misfit function. Both the ammonium oxidation (O_{NH4}, i.e. gross autotrophic nitrification) and the total gross nitrification rate (O_{NH4}+O_{Nrec}) decreased strongly (0.26 to 0.20x) in the 0-5 cm soil layer of the invaded areas, and moderately (0.61 to 0.79x) in the 5-10 cm layer. These rates decreased with depth in native soils, while the reverse was true in invaded soils (Fig. 5c).

In most soils, the immobilization of NO_3^- to recalcitrant N (I_{NO3}) was not well modelled by *Ntrace* and it had to be discarded because its probability density functions (PDFs) did not follow a normal distribution and its sampling accuracy (monitored with the Gelman's R test) was not acceptable; moreover, the inclusion of I_{NO3} in the model did not improve the misfit function. The I_{NO3} rate was only modelled in Sálvora soils under *C. edulis*, even being the dominant gross NO_3^- consumption process in its surface layer (Fig. 5d). Conversely, the dissimilatory reduction of NO_3^- to NH_4^+ (D_{NRA}) was the exclusive gross NO_3^- consumption process in the other soils (Fig. 5d), strongly decreasing in invaded topsoils (0.07 to 0.04x), and to a lesser extend in invaded deep soils (0.18 to 0.70x).

In *C. edulis* invaded soils (and in Nariga deep soil under native vegetation) gross immobilization was the dominant NH_4^+ consumption process, exceeding its oxidation rate by 1.18-2.42x, especially in the surface layer, being found the opposite trend in the other native soils (0.55 to 0.21x). Gross production of NO_3^- always exceeded gross consumption, both in uninvaded and invaded soils (3.22 to 31.05x). Gross consumption of NH_4^+ exceeded its gross production (1.23 to 1.78x), except for the surface invaded soils (0.82 to 0.94x).

3.3. Net N fluxes

There was not a clear effect of *C. edulis* invasion on the net ammonification rate, but the net nitrification rate decreased in invaded soils, especially in surface soils (0.18 to 0.32x). Overall, net mineralization moderately decreased in invaded soils (0.32 to 0.69x), except for the light decrease in Sálvora deep soil (0.94x).

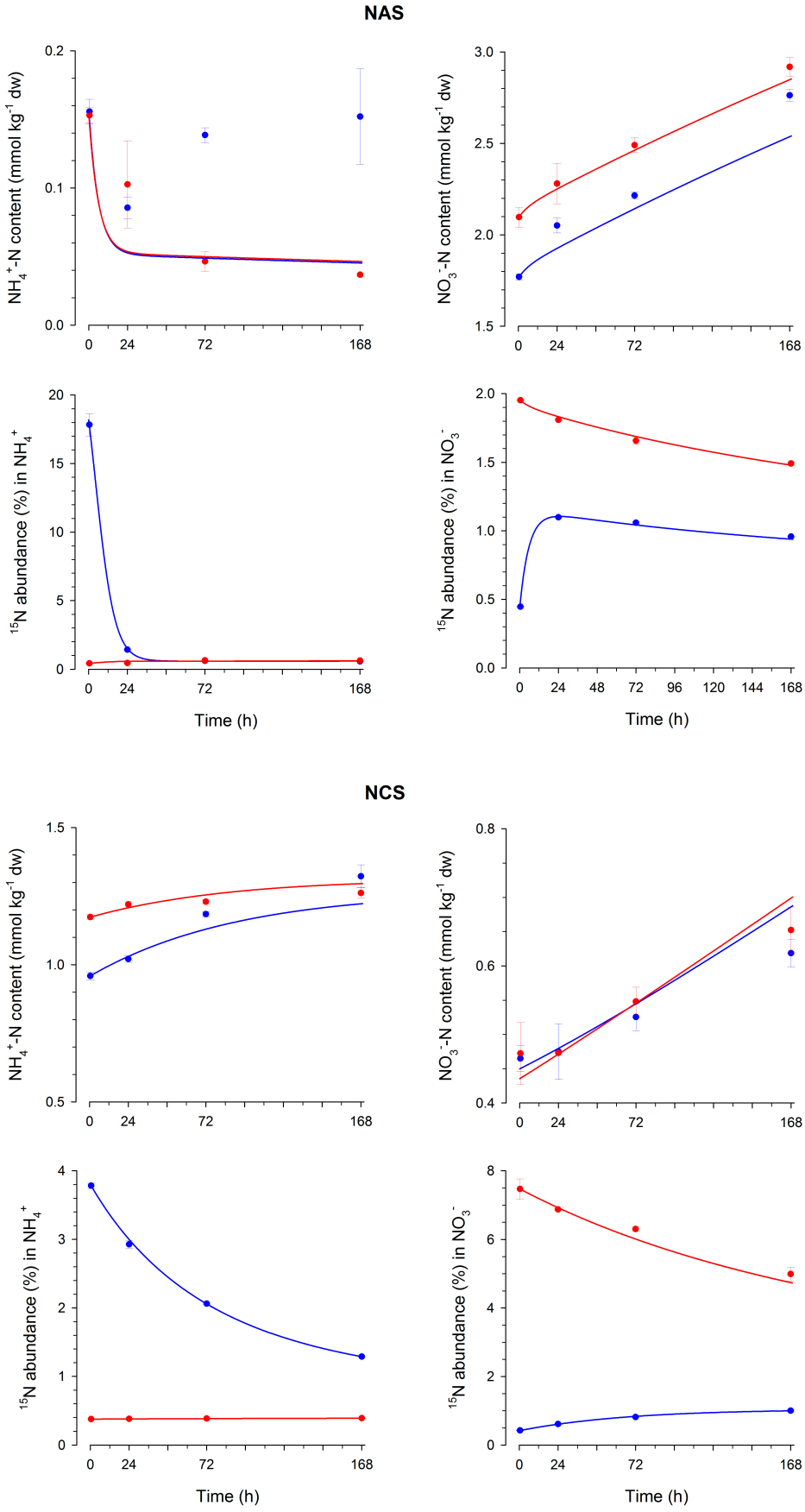


Fig. 1. Concentration and ^{15}N abundance of NH_4^+-N and NO_3^--N (mean ± standard error) in the 0-5 cm layer of Nariga soils, under autochthonous vegetation (NAS) and *Carpobrotus edulis* (NCS), during the aerobic incubation (0.5, 24, 72 and 168 h). Lines show the fit of the model to the experimental data. Blue points or lines refer to the $^{15}NH_4NO_3$ experiment, whilst red points or lines refer to the $NH_4{}^{15}NO_3$ experiment.

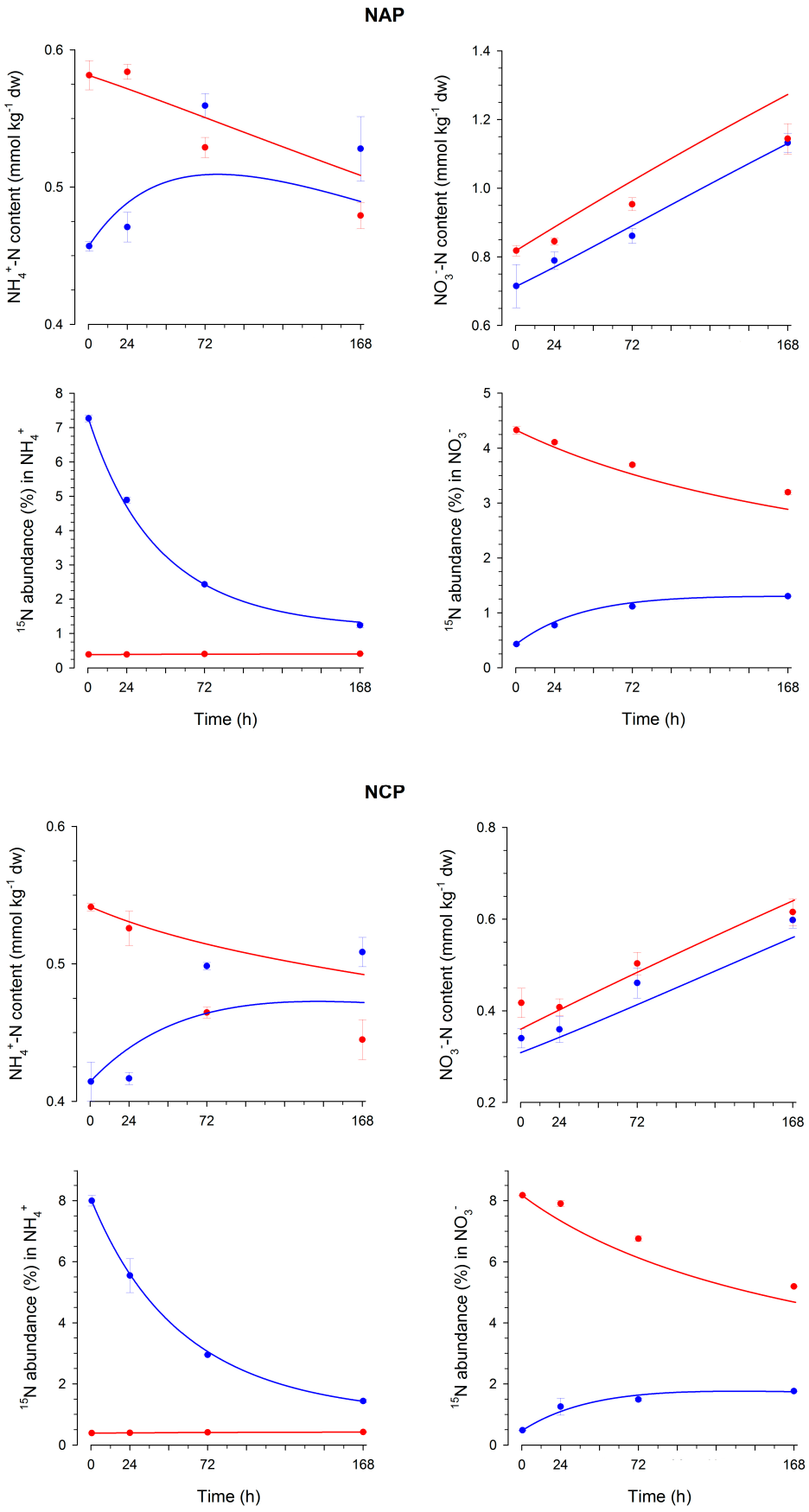


Fig. 2. Concentration and ^{15}N abundance of NH_4^+-N and NO_3^--N (mean ± standard error) in the 5-10 cm layer of Nariga soils, under autochthonous vegetation (NAP) and *Carpobrotus edulis* (NCP), during the aerobic incubation (0.5, 24, 72 and 168 h). Lines show the fit of the model to the experimental data. Blue points or lines refer to the $^{15}NH_4NO_3$ experiment, whilst red points or lines refer to the $NH_4{}^{15}NO_3$ experiment.

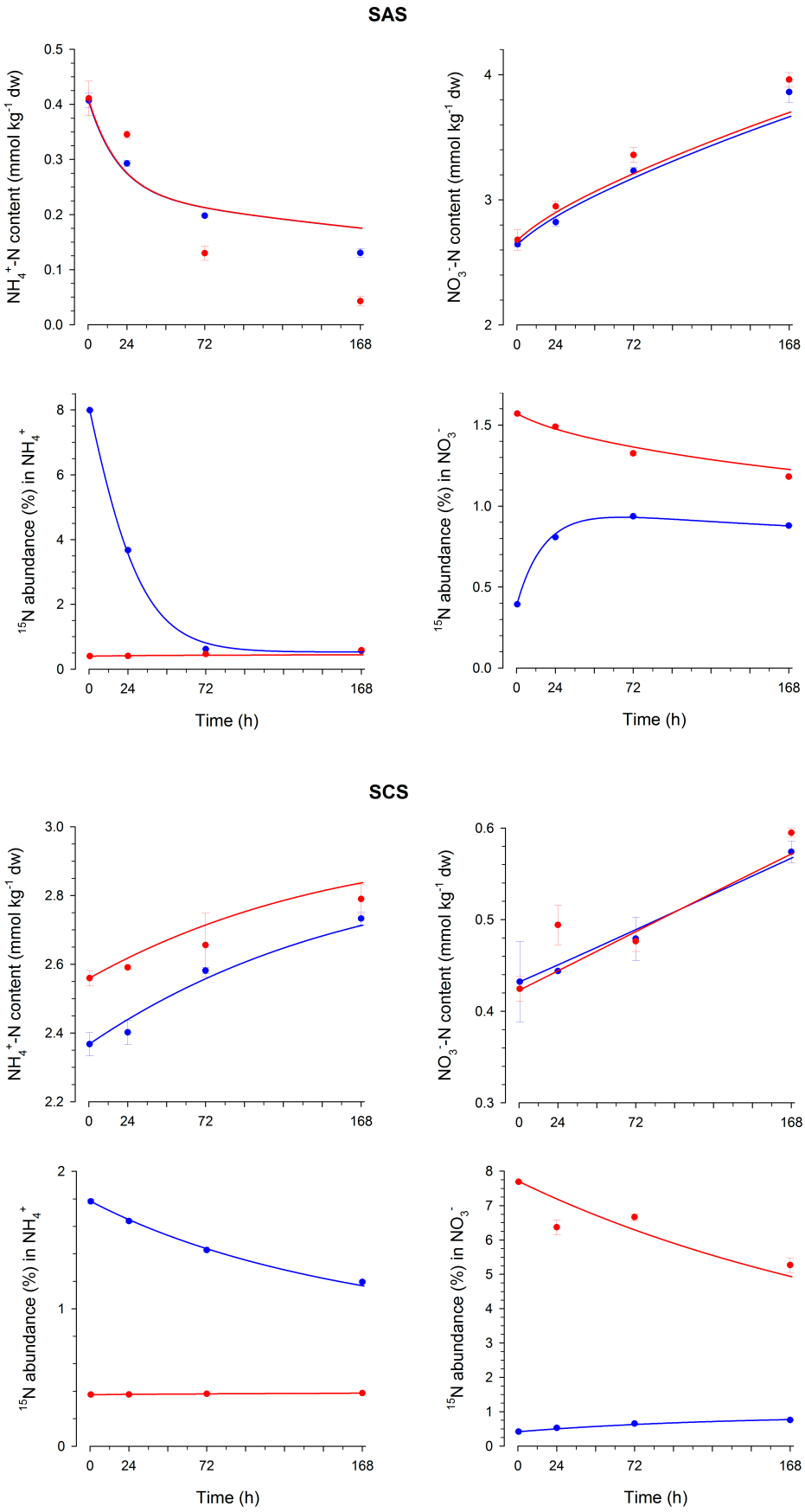


Fig. 3. Concentration and ^{15}N abundance of NH_4^+-N and NO_3^--N (mean ± standard error) in the 0-5 cm layer of Sálvora soils, under autochthonous vegetation (SAS) and *Carpobrotus edulis* (SCS), during the aerobic incubation (0.5, 24, 72 and 168 h). Lines show the fit of the model to the experimental data. Blue points or lines refer to the $^{15}NH_4NO_3$ experiment, whilst red points or lines refer to the $NH_4{}^{15}NO_3$ experiment.

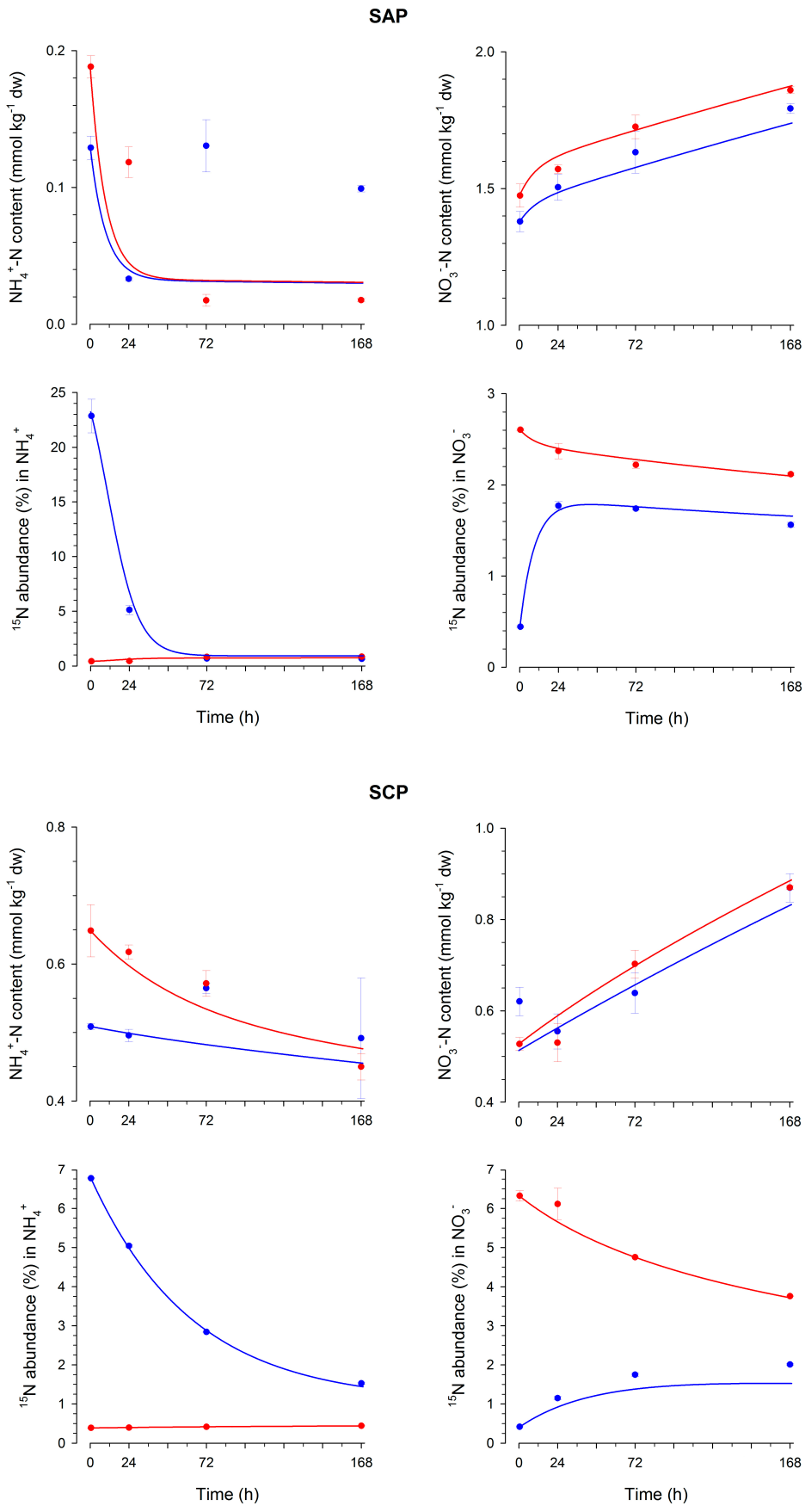


Fig. 4. Concentration and ^{15}N abundance of NH_4^+-N and NO_3^--N (mean ± standard error) in the 5-10 cm layer of Sálvora soils, under autochthonous vegetation (SAP) and *Carpobrotus edulis* (SCP), during the aerobic incubation (0.5, 24, 72 and 168 h). Lines show the fit of the model to the experimental data. Blue points or lines refer to the $^{15}NH_4NO_3$ experiment, whilst red points or lines refer to the $NH_4{}^{15}NO_3$ experiment.

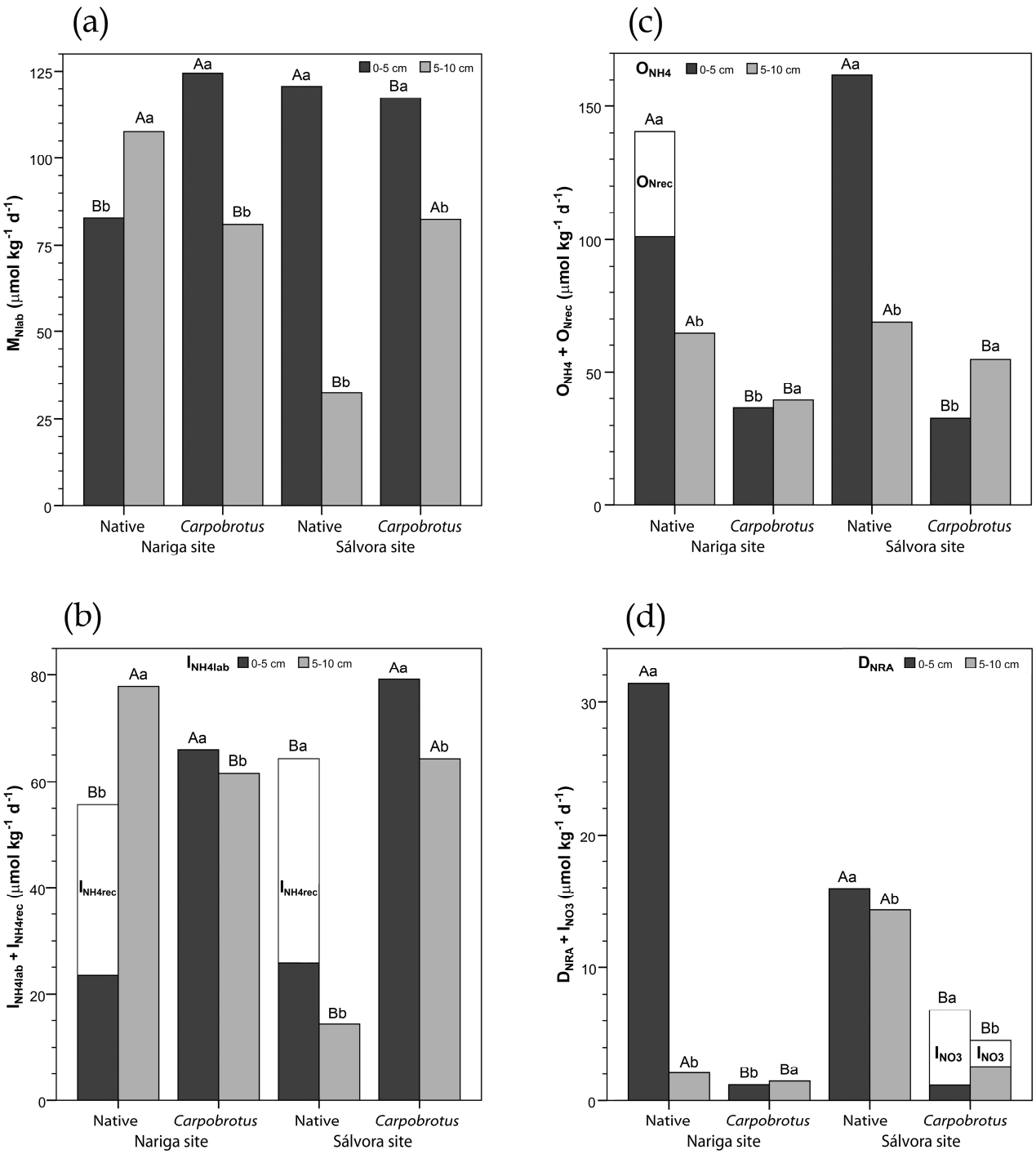


Fig. 5. *Ntrace* modelled gross fluxes in the 0-5 cm and 5-10 cm soil layers under native vegetation and *Carpobrotus edulis* in Nariga and Sálvora sites: (a) mineralization of labile SON to NH_4^+ (M_{SONlab}); (b) immobilization of NH_4^+ to recalcitrant (I_{NH4rec}) and to labile SON (I_{NH4lab}); (c) autotrophic (O_{NH4}) and heterotrophic (O_{SON}) nitrification; and (d) dissimilatory nitrate reduction to ammonium (D_{NRA}) and NO_3^- immobilization to SON (I_{NO3}). Statistical significance in differences - tested by an overlap of the 85% confidence intervals (Payton *et al.*, 2000; Rutting *et al.*, 2010) - is shown by different capital letters for the types of vegetation (native or *Carpobrotus*), and lowercase letters for soil layers (0-5 cm or 5-10 cm).

4. Discussion

In all soils, gross N mineralization was fully explained by the mineralization of the labile pool (M_{SONlab}), while that of the recalcitrant pool (M_{SONrec}), always badly modelled, was not necessary in the models. This result agrees with the existence of a labile (mostly rhizospheric) N pool almost independent of a recalcitrant one (strongly stabilized by aluminium in organomineral complexes), as suggested by González-Prieto and Carballas (1991) on the basis of an acid hydrolysis study of soil organic N in the same region. No clear effect of the invasion was found in gross N mineralization and net ammonification. Studies on the effect of invasive plants on gross N mineralization are scarce, and mostly focused on invasive annual grasses invading areas with native perennial grasses (Booth et al., 2003; Hawkes et al., 2005; Parker and Schimel, 2010; Piper et al., 2015; Stark and Norton, 2015). Annual and perennials plants differ in many traits (phenology, longevity, rooting system, litter characteristics, relationships with soil microbiota) that influence N cycle in the soil-plant system (Parker and Schimel, 2010); as a result, such studies usually reported increases on N mineralization due to the lower C/N ratio of the annual invaders litter and to their higher plant productivity. Nevertheless, in our study case the C/N ratio of the litter from invaded areas is almost identical to that of the native areas (Vieites-Blanco and González-Prieto, 2017) and both the alien plant and the native species are perennial, either grasses as *Armeria pubigera* or (sub)-shrubs as *C. edulis* and *Ulex europaeus*.

Carpobrotus edulis invasion enhanced NH_4^+ immobilization, except in the deep layer of the most recently invaded soil, Nariga, where it slightly decreased. As even small changes in organic matter can largely affect decomposition rates and net N mineralization, an alteration in C inputs in invaded soils due to a change towards a more productive plant species can promote microbial N immobilization (Knops et al., 2002). Higher microbial immobilization was also found in other plant invasions, attributed to a higher microbial activity or plant C inputs (Bengtsson et al., 2003; Hawkes et al., 2005; Laungani and Knops, 2012). However, no effect of *C. edulis* in the organic C content of our soils, but a 4-fold increase in the accumulated necromass, were previously found (Vieites-Blanco and González-Prieto, 2017), suggesting a slow mineralization of *C. edulis* necromass, likely due to its recalcitrance to decomposition (Conser and Connor, 2009; Novoa et al., 2014); these factors likely led to a shortage of available N and the increased microbial N immobilization we observed.

The lower autotrophic nitrification rates (O_{NH4}) we found in soils under *C. edulis* pointed to an effect over the activity and composition of soil microbiota (Knops et al., 2002). Most studies about the effect of invasive plants on gross nitrification had shown either no changes (Laungani and Knops, 2012) or increased rates, most likely due to changes in microbiota composition and abundance (Booth et al., 2003; Hawkes et al., 2005) or plant coverage (Parker and Schimel, 2010). The decrease we found in gross nitrifying rates could be related to an inhibition of nitrifying bacteria by secondary metabolites from the alien plant [like found for *Centaurea stoebe* L. by Thorpe and Callaway (2011)] or to its indirect effect over soil properties. Although a raise of C/N ratio can constrain nitrification (Bengtsson et al., 2003; Laughlin, 2011), the slight increase reported in our soils [from 16.4 to 17.1, Vieites-Blanco and González-Prieto (2017)] seems insufficient to explain the strong reduction of O_{NH4} we found. A more likely explanation is the strong acidification (-1.2 pH units) triggered by *C. edulis* invasion (Vieites-Blanco and González-Prieto, 2017) which drops soil pH below the optimum for nitrifiers (Prosser, 1990). Similar results would be expected in other *C. edulis* invaded areas, because soil acidification is the most widely reported effect in both rocky and dune ecosystems, although no changes in soil pH or slight increases have also been found in dunes (Vieites-Blanco and González-Prieto, 2017). The higher rate of NH_4^+ immobilization (I_{NH4}) compared to O_{NH4} found in all invaded soils (and in Nariga uninvaded deep soil), indicates that heterotrophic bacteria outcompeted nitrifying microbiota for NH_4^+, as also pointed out by Laungani and Knops (2012) and Bengtsson et al. (2003).

Despite being incubated in aerobic conditions, the dissimilatory reduction rate of NO_3^- (D_{NRA}) was the only NO_3^- consuming process in six out of eight studied soils: Sálvora soils under native vegetation and all Nariga soils; moreover, it was also higher than I_{NO3} in the Sálvora topsoil under *C. edulis*. Although D_{NRA} was previously considered an anaerobic process (Pandey et al., 2016; Rütting et al., 2011; Tiedje et al., 1984), it seems to be less sensitive to oxygen exposure than expected (Pett-Ridge et al., 2006). In the last years, there are growing evidences

that D_{NRA} is much more frequent than previously though (Rütting et al., 2011), being even the dominant or exclusive NO_3^- consuming process in N-limited ecosystems with high precipitation rates which provide adequate conditions for DNRA (Huygens et al., 2007; Zhang et al., 2011). Moreover, Pett-Ridge et al. (2006) reported higher D_{NRA} in aerobic soils than in anoxic or alternating oxic-anoxic soils, a result which can be explained by the co-occurrence in aerobic soils of higher NO_3^- levels and D_{NRA} activity in anaerobic microsites [see also Rütting et al. (2011)]. It is well known that the strong mineralization activity in soils rich in OM or amended with organic residues can deplete O_2 levels in the soil atmosphere, leading to anaerobic microsites especially inside soil aggregates (Norton and Stark, 2011; Tiedje et al., 1984). This can likely be the case in our soils which had high C content [11.5-14.1% and 5.5-7.7% in the 0-5 and 5-10 cm layers, respectively, Vieites-Blanco and González-Prieto (2017)] and especially in soils under native vegetation, where more substrate for D_{NRA} is available due to high O_{NH4} rate and NO_3^- levels. D_{NRA} decreased in *C. edulis* invaded soils, which could be explained by the lower substrate availability in these soils, being this effect more accused in the 0-5 cm layer, where both the NO_3^- concentration (Figs. 1-4) and O_{NH4} are the lowest. A decrease in D_{NRA} would also be expected in other *C. edulis* invaded areas, taking into account the previously discussed negative relationship between O_{NH4} (the main producing rate of NO_3^-, i.e., the substrate for D_{NRA}) and acidification of invaded soils. However, as other studies have found a positive relation of D_{NRA} with C/NO_3^- ratio and organic C (Chen et al., 2015; Lu et al., 2015), a direct negative impact of *C. edulis* invasion on microbial D_{NRA} activity cannot be discarded.

Both net nitrification and net mineralization showed lower rates in invaded soils, contrarily of what was found in other studies where microbial activity seems to be potentiated by the alien plants (Parker and Schimel, 2010; Piper et al., 2015; Stark and Norton, 2015). The increase in mineralization rates found in most of the studies on plant invasions seems to be related to the higher N content and lower C/N ratio found in the invaders litter (Lee et al., 2017). However, in our studied areas these characteristics do not apply to *C. edulis* litter, which has slightly higher C/N ratio and slightly lower N concentration than natives litter (Vieites-Blanco and González-Prieto, 2017). The decrease on net N mineralization in the invaded soils could limit N availability; therefore, effects on plant community composition cannot be discarded [see Eviner and Chapin III (2003)].

Although stronger effects of *C. edulis* were found on the 0-5 cm than in the 5-10 cm layer for D_{NRA}, O_{NH4} and O_{NH4}+O_{Nrec}, these N fluxes were affected by the invasion in both layers, contrarily to the effects of *C. edulis* in soil physicochemical properties, which are often confined to the superficial layer (Vieites-Blanco and González-Prieto, 2017).

Overall, the present study on the effect of *C. edulis* on N fluxes, and previous studies on its effect on N stocks (Novoa et al., 2013; Novoa et al., 2014; Vieites-Blanco and González-Prieto, 2017), showed that this alien plant can impact the N cycle of the invaded ecosystems. Of particular concern would be potential legacy effects of *C. edulis* on the N cycle [as seen for other species (Elgersma et al., 2011)], which could affect ecosystem restoration and plant succession. Consequently, we considered that studies on the functioning of the N cycle after *C. edulis* removal would be of high importance for monitoring restoration success.

5. Conclusions

Through its effect over soil physicochemical properties and microbiota, the invasion of rocky coastal habitats by *C. edulis* impacts the N cycle both by changing the pools and fluxes (gross and net) of N in the 0-5 cm and 5-10 cm soil layers. In the invaded soils the NO_3^--N pool decreases, while the NH_4^+-N pool increases. The main NH_4^+ consuming process was immobilization (I_{NH4}) in invaded soils and autotrophic nitrification (O_{NH4}) in native soils. These results were likely explained by an invasion-triggered reduction of N availability, which increased I_{NH4}, and the inhibition of nitrifiers activity due to soil acidification or *C. edulis* exudates, which decreased O_{NH4}. Despite its usual association to anaerobic conditions, the dissimilatory reduction to ammonium (D_{NRA}) was the exclusive or dominant NO_3^- consumption rate in most of our C-rich soils. The oxygen depletion in microsites usually found in soils rich in (or amended with) organic matter could create the adequate conditions for D_{NRA}. The invasion reduced D_{NRA}, possibly due to the lower NO_3^- availability of this soils. Overall, net mineralization decreases in invaded soils, which limits soil N availability to plants, with unknown consequences on plant community composition.

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