Microbial recycling of dissolved organic matter confines plant nitrogen uptake to inorganic forms in a semi-arid ecosystem

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Abstract

Plant uptake of dissolved organic nitrogen (DON) has been proposed to explain inconsistency in the ecosystem N balance of semi-arid systems. Nevertheless, direct evidence for an ecologically important role of DON in plant nutrition in these systems remains elusive under field conditions. Here, natural abundance ^{15}N signatures of NO_3^- , NH_4^+ , DON and whole plants from a semi-arid model forest were analyzed to provide robust estimates of plant N source partitioning and relative N cycling rates under *in-situ* conditions. Concurrently, architectural and symbiotic root traits were determined to assess their relationship to plant N acquisition strategies. Bayesian isotope mixing models indicated an insignificant contribution of DON to ecosystem plant N nutrition. Nitrate was the dominant plant N source in this ecosystem, while the contribution of NH_4^+ to plant nutrition varied between herbaceous (26%) and woody plants (8%). The low C:N ratio of the dissolved organic matter pool - ranging from 12.7 to 4.9 within the soil profile - indicated microbial C-limitation in this ecosystem. Dissolved organic N was significantly enriched in ^{15}N relative to NH_4^+ and NO_3^- , corroborating the importance of dissolved organic matter recycling as a cost-effective pathway that simultaneously supplies C and nutrients for microbial metabolism. Plants exclusively depend on inorganic N forms made available through microbial N mineralization and free-living atmospheric N_2 fixation, followed by autotrophic nitrification.

Keywords: biological nitrogen fixation; Chaco; dissolved organic nitrogen; nitrification; plant-microbial competition.

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1. Introduction

Assessing patterns of plant nitrogen (N) nutrition is important given its impacts on plant biochemistry and physiology, and its consequences for the structuring of plant communities (Lambers et al., 2008; McKane et al., 2002). Historically, it was anticipated that plants depend entirely upon inorganic N that is available after microbial N demands are met (Schimel and Bennett, 2004). Nevertheless, since the 1990s, it became increasingly clear that depolymerisation of N-containing soil organic matter complexes releases organic N monomers that can be taken up directly by plants (reviewed in Näsholm et al., 2009). Especially in N-limited arctic, alpine and boreal ecosystems, it has been documented that dissolved organic N (DON) makes up a significant share of the plant N sources (Chapin et al., 1993; Lipson et al., 1999; Näsholm et al., 1998). Yet, these conclusions cannot consistently be extrapolated to other ecosystems as subsequent studies have confirmed that inorganic N is the dominant plant N source in ecosystems where N is not a limiting element for plant growth (Finzi and Berthrong, 2005; Harrison et al., 2007; Jones et al., 2013). The mechanisms underlying the different patterns of plant N uptake across ecosystems remain largely unclear, but have been hypothesized to relate to relative availabilities of inorganic versus organic N forms in the soil matrix, and patterns of plant-microbial competition (Houlton et al., 2007; Näsholm et al., 2009).

There are several lines of evidence indicating the need for detailed studies of the role of DON in semi-arid ecosystems with summer precipitation. Firstly, plant N-limitation is a widespread phenomenon in these systems (Yahdjian et al., 2011). The N supply and N demand by plants and microbes may be discontinuous and temporally asynchronous in arid and semiarid ecosystems systems. Nitrogen is readily available at higher concentrations during the time plants and microbes are relatively inactive due to dry soil conditions (Austin et al., 2004), but the large

increases in plant N uptake and bursts of microbial activity rapidly exhaust the available N when water stress is alleviated (Evans and Burke, 2013). The extent of plant N-limitation in semi-arid ecosystems is demonstrated in a meta-analysis including 68 independent N fertilization experiments that cover a 50-650 mm yr⁻¹ precipitation gradient (Yahdjian et al., 2011). The highest degree of plant N-limitation was observed in sites where plant water stress during the plant growing season was suppressed, that is, in relatively more humid sites and in those with summer precipitation (Yahdjian et al., 2011). Secondly, the total gross production of DON and the enzymatic potential for proteolysis relative to ammonification is much higher in semi-arid soils than in other ecosystems, including boreal ecosystems (Hofmockel et al., 2010). Potentially, the fact that the optimal pH for protease activity (neutral to alkaline; Ladd, 1972) is similar to the pH values found in semi-arid ecosystems, and the high abundance of proteolytic fungi in these soils (Collins et al., 2008) may stimulate soil organic matter depolymerisation. Additionally, the recurrent drying-rewetting cycles during the wet season induce a continuing input of amino acids as microorganisms release internal solutes, such as free amino acids and their derivatives, that were accumulated to maintain the internal water potential in balance with the surrounding environment during dry periods (Borken and Matzner, 2009; Schimel et al., 2007). Thirdly, incongruities have been observed between measured rates of production of inorganic N and annual ecosystem N budgets in semi-arid ecosystems (Manzoni et al., 2006; Mobley et al., 2014). These findings suggest that plants may take up DON without relying on microbial mineralization in these systems (Mobley et al., 2014). Fourthly, plants from arid and semi-arid ecosystems have been repeatedly reported to have the capacity to acquire organic N such as amino acids via root uptake (Chen et al., 2015; Chen et al., 2010; Hawkins et al., 2005; Jin and Evans, 2010).

A major argument against organic N substantially contributing to plant N nutrition in semi-arid ecosystems is the strong competition for N with soil microorganisms (Chen et al., 2015). The larger surface to volume ratios of microbes and their fast turnover rates may give them a competitive advantage over plant roots for scavenging organic N in the soil (Kuzyakov and Xu, 2013; Näsholm et al., 2009). Farrell et al. (2014) indicated that microbial communities especially assimilate dissolved organic matter to fulfil their carbon (C) requirement, suggesting that plant DON uptake may be primarily restricted under conditions of microbial C-limitation. However, the implications of these findings for semi-arid systems remain unknown due to the extensive spatiotemporal variation in limiting resources that might constrain microbial activity (Pointing and Belnap, 2012; Schaeffer et al., 2003). Moreover, plant root traits have a direct control over the extent of plant-microbial competition (Moreau et al., 2015). Plants can actively control microbial metabolism by changing root mass, rhizodeposition and N uptake rate (Blagodatskaya et al., 2014). Some plants, often being the more fast-growing, resource-acquisitive species in the community, can release large amounts of rhizodeposit-C into the soil that remove microbial C-limitation in semi-arid soils (Fisk et al., 2015; Paterson, 2003). Branching intensity is a major important root parameter as the zone immediately behind the root tip is the site of highest root exudation and nutrient uptake (Häussling et al., 1988; Neumann, 2007) (Marschner, 2011). At the same time, highly branched root systems are characterised by large amounts of fine roots and high specific root lengths that are associated to the efficient uptake of less mobile N compounds from the soil (Bardgett et al., 2014; Cambui et al., 2011; Wardle et al., 2004).

Here, we assess the relative plant uptake of organic and inorganic N under *in situ* conditions over a time-integrated period for a semi-arid model ecosystem. We determined preferential N uptake for all dominant plants in the ecosystem in order to account for presumed

variations in root traits and plant nutrient acquisition strategies. The methodological approach applied involved natural abundance ^{15}N measurements ($\delta^{15}\text{N}$) of compound-specific soil N sources, root, shoot and leaf material. On the strict condition that the isotopic abundance significantly differs among potential plant N sources, this method provides time-integrated information on preferential N uptake patterns under undisturbed plant rhizosphere conditions and natural resource availabilities. Intrinsically, this approach has the potential to overcome the methodological limitations that call into question the validity of many previous assessments of plant N source partitioning (Jones et al., 2013; Näsholm et al., 2009). We hypothesized that DON uptake is an ecologically important pathway for plant N nutrition in this semi-arid ecosystem, especially for plant species that dispose of a highly branched root system with a great specific root length.

2. Materials and methods

2.1 Study site and experimental design

The Natural Reserve of Chancaní, located in the southern extreme of the South American Gran Chaco, in central-western Argentina (ca. $31^{\circ}17' - 31^{\circ}50'$ S and $65^{\circ}16' - 65^{\circ}32'$ W) was used as a model system. The natural reserve encompasses 4960 ha of well-preserved Chaco xerophytic forest, with the trees *Aspidosperma quebracho-blanco* Schltdl. and *Prosopis flexuosa* DC. as canopy and subcanopy dominants, respectively. The shrub layer is dense and dominated by *Mimozyanthus carinatus* Griseb., *Acacia gilliesii* Steud. and *Larrea divaricata* Cav. (Conti and Díaz, 2013). Total stand biomass is 105 Mg ha^{-1} , with a tree, shrub and herbaceous cover of 44%, 79% and 70%, respectively (Conti and Díaz, 2013). The area is characterized by a continental, mesothermal, semiarid climate with dry winters (Capitanelli, 1979). Rainfall oscillates between

300 and 600 mm per year. Mean annual temperature is 21°C and annual potential evapotranspiration ranges between 1000 and 1200 mm. Soils are sandy-loam Aridisols of alluvial origin, characterized by a neutral pH (pH_{H2O}: 7.0), a bulk density of 1.4 g cm⁻³, and topsoil (0-10 cm) organic C contents, inorganic C content and N contents of 16.3 g kg⁻¹, 1.97 g kg⁻¹, and 1.69 g kg⁻¹, respectively (Conti and Díaz, 2013; Conti et al., 2014). Four replicate forest plots (50 x 50 m) were established in the reserve, with an average distance of 1.2 km between plots.

Three composite (subreplicate) soil samples, each made up of 6 subsamples, were collected in each of the 4 replicate plots using a soil auger in January 2014 for $\delta^{15}\text{N}$ measurements of plant N sources. Normal climate conditions were recorded for the time of sampling, with a total precipitation of 207 mm and average temperature of 25°C in the 4 months prior to the field campaigns (data for Villa Dolores, located at ~80 km from the study sites). Samples were taken up to 60 cm deep in order to collect soil material from the root nutrient depletion zones of shallow and deep plants (7 soil depth intervals: 0-5 cm, 5-10, 10-15 cm, 15-20, 20-30 cm, 30-40 cm, and 40-60 cm). Soil samples were stored cool and returned to the laboratory after which roots were separated from soil. Soil extractions took place within 48h after sampling. A soil subsample was dried for bulk soil $\delta^{15}\text{N}$ analysis (single composite sample per plot replicate). Soil extracts were shipped frozen to Ghent University and analyzed for $\delta^{15}\text{N}$ of NH_4^+ , NO_3^- and DON (three subreplicates per plot replicate).

Sixteen woody (10) and herbaceous (6) species were excavated during two field campaigns that took place in the Austral summer (December 2013 – January 2014) (Table 1). The species were selected in order to cover a wide range of resource acquisition strategies and belonged to ten different families. Altogether, the species sampled make up 82% of the total plants in the forest plant community (based on counting of plant individuals), with relative

abundances varying from 15.3% (*Mimozyanthus carinatus* (Griseb.) Burkart) to 0.2% (*Selaginella sellowii* Hieron.; Table 1). For each species, three individuals were sampled in each of the four replicate forest plots (true replicates, $n = 4$), resulting in a total of 192 plants. Adult individuals were selected for herbaceous plants, while saplings with an aboveground height of about 70-90 cm were sampled for woody trees and shrubs. Plant roots were manually excavated using a pitchfork and smaller gardening tools such as a mini-spade, mini-pick, palette knife and trowel. For some deep rooted individuals, plant root systems were cut at a depth of 60 cm as a hard soil layer hampered deeper excavation. Drawings were made in the field to describe some general aspects of the plant root architecture, such as the depth and the orientation of seminal and first order roots. The plant material was stored in paper bags, and brought to the laboratory for further processing within 5 days. Shoot and root biomass was separated and, if necessary, washed to remove any root-adhering soil material. Both parts were dried at 40°C until constant weight and mass of root, stems and leafs were recorded. From each species and replicate plot, the first individual was used for $\delta^{15}\text{N}$ and total nitrogen (TN) measurements of leaves, twigs, stems, coarse roots (>1 mm) and fine roots (< 1 mm); coarse and fine roots were separated per depth interval (0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm, 40-60 cm). Fine roots of the second individual were separated in subsamples for $\delta^{15}\text{N}$ and TN determinations, mycorrhizal root colonization and C measurements. The remaining part of the second individual (coarse roots and shoot) was ground for $\delta^{15}\text{N}$ and TN analysis. The third individual was used for root architecture determinations.

2.2 Plant nutrient and isotope measurements

Controversy to the use of $\delta^{15}\text{N}$ as an *in situ* tracer is also associated to the sampling of specific plant organs, such as leaves, that may show large deviations from whole plant signals due to internal plant processes (Pardo et al., 2013; Robinson, 2001). Therefore, Robinson (2001) recommended to sample whole plant biomass, including root systems. Here, all plant parts of the first and second individual per replicate plot were weighted and analyzed to enable whole plant balances. Parts with a large biomass were ground using a cutting-mill system having a 250 μm sieve (MF 10, IKA, Germany). Small weight plant samples were ground to fine powder using a planetary ball mill, with further processing using a pestle and mortar for homogeneous sample achievement when necessary. Total N (TN) and $\delta^{15}\text{N}$ was measured on all plant tissue samples. Additionally, total carbon (TC) was determined for fine root samples. Finally, we disposed, for each of the four replicate forest plots ($n = 4$), of two sub-replicates for whole plant $\delta^{15}\text{N}$ values, single sub-replicate for $\delta^{15}\text{N}$ and TN values of leaf, twig, stem, and fine and coarse roots separated per depth interval, and single sub-replicates for fine root TC.

2.3 Soil extraction, N concentrations and nitrous oxide conversions of dissolved N pools

Fresh soil (~ 650 g) was divided into 2 subsamples for bulk soil $\delta^{15}\text{N}$ and soil moisture determination, and KCl extraction. The moisture content was determined by drying an approximate, known amount of ~ 50 g dried at 40°C until constant weight, after which the sample was used for bulk soil $\delta^{15}\text{N}$ measurements. The remaining soil (~ 600 g) was extracted using an approximate, known extraction ratio of about 1:2 (w/v; 1 M KCl; end to end shaking at 60 rpm for 60 min.). Extracts were finally filtered over a 0.45 μm filter (GD/X syringe filters, Whatman, USA). The N concentrations and $\delta^{15}\text{N}$ of dissolved N pools was determined using standard protocols described in Huygens et al. (2013). In brief, concentrations of NH_4^+ and NO_3^- were

measured using wet chemistry segmented flow analyser (Bran+Luebbe, Germany) and standard colorimetric techniques (Kirkwood, 1996). The total dissolved N (TDN) was digested using alkaline persulfate digestion (Doyle et al., 2004) and measured as NO_3^- . Extractable DON was estimated as the difference between the concentrations of TDN and dissolved inorganic N. For the measurement of isotopic signatures, NH_4^+ , NO_3^- and DON were converted to N_2O . Nitrate was converted into N_2O using a denitrifier (*Pseudomonas aureofaciens*; ATCC 13985) lacking the N_2O reductase. Ammonium was converted to N_2O using ammonia microdiffusion (Holmes et al., 1998), followed by persulfate oxidation of recovered NH_4^+ onto a glass fiber filter and the denitrifier method. Total dissolved N conversions were performed using persulfate oxidation followed by the denitrifier method.

2.4 Analytical analyses and natural abundance isotopic calculations

The isotopic signatures of nitrous oxide converted NH_4^+ , NO_3^- and DON were measured using an IRMS coupled to pre-concentration unit (Precon) and gas chromatograph (ANCA-TGII, PDZ Europa, UK). Equipment precision is better than $\pm 0.2\text{‰}$ for $\delta^{15}\text{N}$. All isotopic values were corrected for N species in blanks (1M KCl) according to Hayes (2004). The ^{15}N enrichment of DON was calculated using the following mass balance equation:

$$F_{\text{DON}} = [(M_{\text{TDN}} \times F_{\text{TDN}}) - (M_{\text{NH}_4^+} \times F_{\text{NH}_4^+}) - (M_{\text{NO}_3^-} \times F_{\text{NO}_3^-})] / M_{\text{DON}} \quad (1)$$

Where M terms represent the N concentration of the N form of interest and the F terms represent fractional isotopic abundances ($F = ^{15}\text{N} / (^{14}\text{N} + ^{15}\text{N})$). For natural abundance $\delta^{15}\text{N}$ measurements, samples with a NH_4^+ -N concentration lower than 0.4 mg L^{-1} in the soil extract were not analyzed as measurement precision could not be guaranteed. In case analytical constraints impeded the determination of isotopic signals of inorganic N for as specific

subreplicate sample, the value was replaced by an average profile $\delta^{15}\text{N}$ value to enable the calculation of the $\delta^{15}\text{N}_{\text{DON}}$ value. Based on previous experiences at ISOFYS, we did not measure samples for $^{15}\text{N}_{\text{DON}}$ enrichment that had an inorganic N/total dissolved N higher than a threshold value of 0.75 to avoid an interference with inorganic species and reduce the cumulative analytical variance of DON measurements (Lee and Westerhoff, 2005).

Extractable dissolved organic C was measured using a total organic C analyzer (Shimadzu 5000 Series, Japan) after removal of inorganic C and by acidification and purging with N_2 during 60 min.

Measurements of $^{15}\text{N}/\text{TN}$ and $^{13}\text{C}/\text{TC}$ of solid plant material were performed in single inlet mode using an elemental analyser (ANCA-SL, PDZ Europa, UK) coupled to an Isotope Ratio Mass Spectrometer (EA-IRMS; 20-20, SerCon, Crewe, UK). Equipment precision is better than $\pm 0.10\text{‰}$ for $\delta^{13}\text{C}$ and $\pm 0.2\text{‰}$ for $\delta^{15}\text{N}$.

The measured isotopic ratios are presented as δ values (‰) vs. the international standard:

$$\delta_{\text{sample}} = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 1000 \text{ (‰)} \quad (2)$$

Where R_{sample} and R_{standard} refer to the ratio of the heavy isotope to light isotope in the measured sample and the standard, respectively. The international standards were atmospheric air ($\delta^{15}\text{N}$) and Vienna Pee Dee Belemnite (V-PDB, $\delta^{13}\text{C}$)

2.5 Isotope source partitioning and data analysis assumptions

The Bayesian stable isotope mixing model mixSIAR was used to estimate the proportions of source contributions (N forms NO_3^- , NH_4^+ and DON) to a mixture (whole plant N) (Stock and Semmens, 2013). MixSIAR can be used to solve underdetermined stable isotope mixing systems with the outputs representing probable solutions. The applied Bayesian framework permits the

incorporation of uncertainties in isotopic signals of the N sources, the plants, and isotopic fractionation factors for which these models realistically convey baseline complexities and communicate honestly data uncertainty (Moore and Semmens, 2008).

The magnitude of isotopic discrimination between plant $\delta^{15}\text{N}$ and source $\delta^{15}\text{N}$ depends on the ratio of demand by assimilatory enzymes to the concentration of the external N supply (Evans, 2001; Kolb and Evans, 2003). A high external N concentration relative to enzymatic plant demand leads to significant isotopic fractionation between whole-plant and source. Therefore, isotopic discrimination factors of 2-3‰ during plant assimilation of readily available NO_3^- have been documented (Evans, 2001; Högberg, 1997; Tcherkez and Hodges, 2008). For N molecules that are readily consumed by plants and microbial communities, no isotopic N fractionation occurs (Högberg, 1997; Kolb and Evans, 2003; Mariotti et al., 1981). In semi-arid ecosystems, NO_3^- is much more available for plant and microbial communities than reduced N forms for which strong biotic competition occurs (Delgado-Baquerizo et al., 2011; Hofmockel et al., 2010). This is even more expressed considering that only a minor fraction of the DON pool is bio-available (Jones et al., 2004; Näsholm et al., 2009). Also in this study, such patterns of relative availability of dissolved N forms were observed (Fig. 1). Hence, source specific uptake and assimilation fractionation factors were set at -2.5‰, 0‰, and 0‰ for plant uptake by non-mycorrhizal plants of NO_3^- , NH_4^+ , and DON, respectively.

As arbuscular mycorrhizal plants are about 2‰ depleted relative to non-mycorrhizal plants (Craine et al., 2009), we assumed that isotopic fractionation factors for mycorrhizal plants were 2‰ further depleted. A plant individual was considered mycorrhizal when the average percentage of summer mycorrhizal root colonization summer was greater than 10%.

It is recognized that the extent and variability of isotopic fractionation during N uptake depends on soil N availability, biochemical uptake pathways and plant species identity (Evans,

2001; Högberg, 1997; Tcherkez and Hodges, 2008). Contrasting patterns of intraplant variation are observed for the different forms, with a greater variation for NO_3^- than for the reduced N forms (Evans et al., 1996). This can be attributed to pathway-specific differences in the site of N assimilation; reduced N is exclusively assimilated in the root, while NO_3^- can be assimilated in the root and the shoot (Cambui et al., 2011; Evans et al., 1996). A standard deviation of 3‰ for NO_3^- was therefore considered in the MixSIAR model to account for the uncertainty associated to the fractionation factor during NO_3^- uptake and assimilation by plants (Mariotti et al., 1980), meanwhile a significant lower standard deviation of 1‰ was used for the fractionation factors of NH_4^+ and DON.

The natural abundance $\delta^{15}\text{N}$ profiles showed finite differences between the four replicate study plots (data not shown). Therefore ‘plot’ was included as a factor in the MixSIAR model and the three subreplicate samples were used to calculate plot-specific $\delta^{15}\text{N}$ average values and standard deviations. As the $\delta^{15}\text{N}$ values of the sources varied as a function of soil depth, $\delta^{15}\text{N}$ source values were calculated based on N source concentrations profiles and vertical root biomass distributions (as determined using the ‘root length density’ function in SmartRoot, see below) for the two plant growth forms (woody and herbaceous plants).

2.6 Root architecture

The root systems of dry and weighted plants were rewetted and stained in a methylene blue (1 g L^{-1}) solution during 24 hours. Afterwards, plants were rinsed in distilled water and dried during 4 hours at ambient temperatures. A rectangular frame encompassing an inner space of $125 \times 60 \text{ cm} \times 0.2 \text{ cm}$ was used for root architecture measurements. Fifteen transparent acrylic plates ($25 \text{ cm} \times 20 \text{ cm} \times 0.2 \text{ cm}$) were placed in the inner space of the frame, after which the plant root

system was spread out on top of these plates. Transparent adhesive tape strips were used to match and fix the *in-situ* root system according to the field drawings. In case of the existence of woody first order roots that impeded the transformation of the 3-D *in-situ* system towards the 2-D space of the frame, first order roots were also cut at the basis using a mini-drill with a wood saw blade accessory (Dremel 3000, Bosch, Germany) before spreading out the entire root system. Once the entire root system was fixed, the borders between all transparent acrylic plates were overlain with a 4 cm wide transparent tape to firmly fix all root material adjacent to the borders. Afterwards, the borders of all individual plates were untied again using a blade knife that disrupts the wide tape and caused the breaking of the roots underneath the tape between two plates. All individual transparent acrylic plates were then scanned at 800 dpi (8 bit grayscale) using a dual lightning scanner having a maximal scan area of 20 cm x 25 cm (Epson V750-M Pro, Epson, USA). A custom-made acrylic frame was fit to the lower glass plate to delimit the exact scan area when working with the dual lightning system. The entire root system was then digitally reconstructed using a photo-editing program (Photoshop 5.0, Adobe System Incorporated, USA). Root architecture, including morphology and topology, was analyzed using the freeware SmartRoot (Lobet et al., 2011). SmartRoot is a semi-automated powerful tracing algorithm (Lobet et al., 2011). Based on the SmartRoot output data following parameters were determined for each of the plant species: specific root length (SRL, cm g^{-1}), root tissue density (RTD, g cm^{-3}), median root diameter (mm), mean root diameter of the first and second order terminal roots (mm) and root branching intensity ($\# \text{ root tips cm}^{-1}$). Root length was determined for each 2.5 cm depth interval using the SmartRoot 'root length density' function. An 'extinction' model was fitted to describe the relationship between cumulative root length and soil depth [cumulative root length = $1 - \beta^d$; with d = soil depth and β the extinction coefficient] (Gale and Grigal, 1987). Plants with a greater

proportion of roots in the deeper soil layers will have higher β value (e.g. 0.98) than plants with a greater root proportion near the surface (e.g. β value of 0.86) (Jackson et al., 1996).

2.7 Mycorrhizal colonization

Fine roots were cleared and stained according to Grace and Stribley (1991). They were mounted in semi-permanent slides in polyvinyl lactic acid-glycerol; we prepared one slide per individual including all active roots. Arbuscular mycorrhizal colonisation (AMF) colonization was determined following the magnified intersection method of McGonigle et al. (1990) using a compound microscope (Nikon optical, Model E200) at 200 $\times$ magnification. Percentage of root was assessed as the proportion of total root intersections that were colonized.

2.8 Statistical analysis

One-way ANOVA analysis of root traits and plant isotopic signatures was performed, followed by Tukey posthoc test. In case of heterogeneity of variances (Bartlett test), a Kruskal-Wallis one-way ANOVA followed by a multiple comparison test was executed. All statistical analyses were performed at the 95% level.

3. Results

3.1 Standing dissolved nitrogen pools and microbial resource stoichiometry

Nitrate was the dominant N form with average concentrations of 12.3 $\mu\text{g N g}^{-1}$ throughout the soil profile (Fig. 1). Standing pools of DON varied from 9.7 $\mu\text{g N g}^{-1}$ to 2.1 $\mu\text{g N g}^{-1}$, and significantly decreased with soil depth. Ammonium concentrations were an order of magnitude smaller and ranged from 2.5 $\mu\text{g N g}^{-1}$ to 0.2 $\mu\text{g N g}^{-1}$ (Fig. 1.). The C:N ratio of dissolved organic

matter showed a value of 12.7 in the 0-5 cm soil layer and decreased with soil depth to 4.9 in the 40-60 cm soil layer.

3.2. $\delta^{15}\text{N}$ values of soil and plant N

The $\delta^{15}\text{N}$ values for whole plants ranged from 1.8‰ to 4.2‰ (Fig. 2a). One-way ANOVA indicated a significant effect of plant species identity on whole plant $\delta^{15}\text{N}$ values (p : 0.04) (Table SI1). Differences between leaf and whole plant $\delta^{15}\text{N}$ values varied from -0.4‰ (*Bromelia urbaniana*) to +2.5‰ (*Capparis atamisquea*) (Table SI1), indicating that the use of leaf material would induce a significant error in calculating N source contributions for the different plants.

The highest $\delta^{15}\text{N}$ values were observed for DON (concentration weighted mean profile value 18.0‰), followed by NH_4^+ (11.8‰) and NO_3^- (5.1‰) (Fig. 2b). The DON $\delta^{15}\text{N}$ values decreased as a function of soil depth from +23.0‰ to +10.5‰, while $\delta^{15}\text{N}\text{-NH}_4^+$ and $\delta^{15}\text{N}\text{-NO}_3^-$ remained largely constant (Fig. 2b). Therefore, the absolute differences between $\delta^{15}\text{N}\text{-DON}$ and $\delta^{15}\text{N}\text{-NH}_4^+$ decreased from 11.9‰ in the topsoil to 5.3‰ in the 15-20 cm soil layer.

3.3 Plant N source partitioning

Bayesian MixSIAR outputs indicated that NO_3^- was the dominant N source for the plants in this semi-arid ecosystem, with an average contribution of 89% and 66% for woody and herbaceous species, respectively (Fig. 3, Table SI2 and SI3). The average source proportion of NH_4^+ to ecosystem N uptake was significantly greater for herbaceous (26%) than for woody species (8%) (Fig. 3, Table SI2 and SI3). The most probable contribution of DON to plant N uptake, as indicated by the median values of the posterior density distributions, were 1.5% and 3.0% for woody and herbaceous species, respectively (Table SI2 and SI3). None of the 16 plant

species took up significant amounts of DON, and no significant differences among N source partitioning patterns were observed between plant species that share a common growth form (Table SI2 and SI3). The matrix plot indicated a strong, negative correlation between NH_4^+ and NO_3^- (-0.93 and -0.78 for woody and herbaceous plants, respectively). Therefore, an increase in the relative contribution of NO_3^- (right hand side of the distribution for NO_3^- in Fig. 3a and 3b) involves thus a decrease in the contribution of NH_4^+ to plant nutrition (left hand side of the distribution for NH_4^+ in Fig. 3a and 3b) and vice versa. The matrix correlations between DON and inorganic N forms were much smaller, indicating that the MixSIAR model is able to provide robust and narrow probability distributions for DON (Fig. 3c and Fig. 3d).

3.4 Plant root traits

There was nearly an order of magnitude of variation in most of the five traits used to assess differences in root morphology and architecture in the 16 plant species (Table 2). Also elemental composition of fine roots varied widely, with the largest differences among observed for fine root N contents (from 0.7% to 2.2%; Table 2). Specific root length, median diameter of first and second terminal order roots, branching intensity and mycorrhizal colonization had the greatest proportional variation among observations. The β values derived from the root extinction model of Gale and Grigal (1987) indicated that woody plants had a greater proportion of their roots located in the deep soil layers, while herbaceous species mainly had their roots near the soil surface (Table 2).

4. Discussion

This study is the first to report quantitative time-integrated information on plant N source partitioning patterns under true *in-situ* conditions for a semi-arid ecosystem. We found that all plant species showed similar N preferences and dominantly relied on NO_3^- for their N nutrition. Dissolved organic N was an insignificant plant N source in this semi-arid model ecosystem. Additionally, the observed $\delta^{15}\text{N}$ patterns of soil N pools and plant biomass provide further insight into the soil N cycle and competitive interactions among plants and microbes for N sources. Specifically, the following N cycling processes are likely to be fundamental to explain the observed isotope patterns (Fig. 4): (1) microbial N uptake patterns, (2) microbial recycling of dissolved organic matter, (3) free-living N_2 fixation, and (4) autotrophic nitrification.

The lack of significant plant DON uptake indicates a greater competitive ability of the soil microbial community to effectively capture organic N sources. Even plants that have a very high specific root length and form a symbiosis with mycorrhizal fungi lose out in the competition with the microbial community for DON molecules. When the C:N ratio of microbial substrates is below a threshold value of about 20-25, their consumers experience energy limitation (Berg and McClaugherty, 2003; Mooshammer et al., 2014). The C:N ratio of dissolved organic matter (ranging from 12.7 to 4.9 throughout the soil profile) observed in this study is thus indicative for strong microbial C-limitations, and likely explains the negligible plant DON uptake patterns in this system. Farrell et al. (2014) showed that soil available C is inversely correlated microbial DON uptake. Under conditions of strong C-limitation, heterotrophic microbial communities predominantly assimilate dissolved organic matter as it is a highly cost-effective pathway that simultaneously supplies C and nutrients for microbial metabolism (Farrell et al., 2014). Although dissolved organic C may be increased in the rhizosphere compared to bulk soil (Koranda et al., 2011), we presume that none of the plants leak sufficient quantities of rhizodeposit-C to

overcome the microbial C-limitation in this semi-arid ecosystem. Our results are in agreement with recent studies that used a methodological approach based on the addition of ^{15}N labelled amino acids to demonstrate that microbial soil communities outcompete plants for DON in semi-arid ecosystems (Chen et al., 2015; Wei et al., 2015). The observed microbial C-limitation is contrary to the findings of Torres et al. (2005), who observed that there was a year-round continuous supply of soluble C to the microbial community present in the plant litter at this study site. There are, however, claims that foliar litter is not readily incorporated into the soil matrix of semi-arid ecosystems due to the limited activity of soil fauna and high photodegradation solar degradation at the ground surface (Austin and Vivanco, 2006; Collins et al., 2008). Additionally, the declining C:N ratios from litter to soil indicate increasing C-limitation along the litter decomposition cascade (Cleveland and Liptzin, 2007; Mooshammer et al., 2014).

The remarkably high $\delta^{15}\text{N}$ enrichment of the DON pool is significantly higher than values observed for boreal, temperate and tropical ecosystems (Houlton et al., 2007; Liu et al., 2013; Mayor et al., 2012; Takebayashi et al., 2010). Under C-limited conditions, microbial communities increase their assimilation efficiency of organic C, leaving soil microorganisms with an internal excess of N to be excreted as NH_4^+ (Farrell et al., 2014; Mooshammer et al., 2014). Isotopic fractionation during microbial N dissimilation and export results in preferential loss of the light ^{14}N isotope, causing high ^{15}N enrichment of the microbial cells relative to its N source (Högberg, 1997; Robinson, 2001). Furthermore, there is now growing evidence that microorganisms are predominantly responsible for the formation, stabilization, and processing of dissolved organic matter (Heinz et al., 2015; Malik and Gleixner, 2013). Malik and Gleixner (2013) showed that dissolved organic matter is a small but reactive fraction of soil organic matter that is composed of microbial necromass and metabolites that are getting constantly decomposed,

altered, or produced anew by soil microorganisms. With each turnover event or successional cycle, N from the previous cycle is retaken up by microbes and the $\delta^{15}\text{N}$ values will further increase due to the proportion of isotopically depleted N that is lost from the organic N cycle as NH_4^+ . The documented $\delta^{15}\text{N}$ enrichments of more than 10‰ for microbial biomass pools relative to their sources observed in other semi-arid woodlands (Dijkstra et al., 2008), corroborate the importance of the high turnover of microbial necromass and metabolites as a key process within the N cycle of semi-arid ecosystems.

The considerably lower $\delta^{15}\text{N}$ value of NH_4^+ compared to DON (up to 12‰ lower in the 0-5 cm soil layer) indicates a significant atmospheric N_2 contribution to NH_4^+ in the topsoil layer. For ecosystems where NH_4^+ is dominantly produced from the microbial mineralization of the DON pool, only small differences between the $\delta^{15}\text{N}_{\text{NH}_4^+}$ and $\delta^{15}\text{N}_{\text{DON}}$ are observed (Houlton et al., 2007; Liu et al., 2013; Mayer et al., 2001; Mayor et al., 2012). This is attributed to the minor isotopic fractionation that takes place during the transformation from organic N to NH_4^+ , and the neutralizing effect of NH_4^+ consumption processes (autotrophic nitrification, NH_4^+ volatilization, and plant uptake) that cause isotopic NH_4^+ enrichment (Högberg, 1997; Robinson, 2001). Another potential source of NH_4^+ in semi-arid ecosystems is atmospheric N_2 fixation by leguminous plants and free-living bacteria (Belnap and Lange, 2003). Many of the dominant plant species in the ecosystem (e.g. *Prosopis flexuosa*, *Mimozyanthus carinatus* and *Acacia gilliesi*) are leguminous. Yet, we did not find N_2 active fixing nodules associated to the sampled plant roots in this study, lending support to earlier findings that symbiotic N_2 fixation may be down-regulated during later stages of forest succession (Batterman et al., 2013). Nevertheless, free-living N_2 fixing bacterial communities are abundant in biological soil crusts of semi-arid ecosystems (Pointing and Belnap, 2012; Torres et al., 2005), and significant leakage of fixed

NH₄⁺ may occur to the surrounding soil environment (Johnson et al., 2007). Because atmospheric N₂ has a δ¹⁵N value of 0‰ and the isotopic fractionation during the N₂ fixation is relatively small (Högberg, 1997), a two-end mixing model with N₂ and DON as end-members indicates a significant contribution of atmospheric N₂ fixation to the topsoil NH₄⁺ pool. In the deeper soil layer, the convergence in isotopic signals between NH₄⁺ and DON shows that a greater proportion of the NH₄⁺ is derived from DON. Though NO₃⁻ is the dominant N source for both woody and herbaceous plants, NH₄⁺ has a greater relative contribution to herbaceous plant N nutrition. This may be linked to their higher relative proportion of roots in the topsoil layers where an increased availability of NH₄⁺ was observed (Fig. 1) (Sala et al., 2012). Another potential reason can be the clear differences in morphological root traits between plant growth forms. The herbaceous plants show a much greater specific root length and branching intensity, root trait patterns that are positively correlated to rhizodeposition (Bardgett et al., 2014). The leakage of labile C compounds in the rhizosphere may provide a competitive advantage for heterotrophic N immobilizers over autotrophic nitrifiers (Fisk et al., 2015), leading to an decreased availability of NO₃⁻ in the rhizosphere of the herbaceous plants in the system. Nevertheless, further research that focuses on rhizosphere-specific patterns of nutrient availability should be undertaken to address the importance of root distribution and root morphology in this process.

Autotrophic nitrification is often a principal NH₄⁺ consuming process in semi-arid soils when available C limits heterotrophic microbial activity (Cookson et al., 2006; Hooker and Stark, 2008). Additionally, the ‘fungal loop’ N cycle model emphasizes the importance of heterotrophic nitrification through protein mineralization in dry ecosystems (Collins et al., 2008). Nevertheless, if the latter process would dominate NO₃⁻ production, one would expect that the δ¹⁵N values of

the newly formed NO_3^- resemble those of DON due to the minor fractionation associated to protein mineralization (Högberg, 1997; Mayer et al., 2001). Rather, it is implied that the somewhat lower $\delta^{15}\text{N}$ value of NO_3^- relative to NH_4^+ is the result of autotrophic nitrification that is accompanied by isotope fractionation effects up to 35‰ depending on the NH_4^+ availability (Mayer et al., 2001). These results are in line with previous studies indicating that autotrophic microorganisms dominate ammonia oxidation and NO_3^- production rather than heterotrophic fungi in semi-arid soils (Marusenko et al., 2013). Moreover, these findings support the synergistic interaction between ammonifiers and N-fixing microorganisms, as suggested by the observed positive correlation between their populations, previously observed at this study site (Torres et al., 2005).

Conclusions

This study documents patterns of plant N uptake and soil N transformations during the plant growing season for a semi-arid model ecosystem. The natural abundance of soil and plant ^{15}N is an integrator of N cycling processes, but requires time-intensive sampling of whole plant biomass and cautious data interpretation as multiple plant and microbial processes interact to determine $\delta^{15}\text{N}$ values (Högberg, 1997). A critical condition to use $\delta^{15}\text{N}$ as a natural tracer for N source partitioning is the occurrence of marked differences in $\delta^{15}\text{N}$ among all potential N source pools (Robinson, 2001). To the best of our knowledge, the range in $\delta^{15}\text{N}$ values for the different N forms observed in our model ecosystem has never been documented before. Our results provide evidence for a tight link between soil C and N cycling in semi-arid ecosystems. We reject our hypothesis that DON is a significant plant N source. Rather, plant N uptake is confined to inorganic N forms because microbial communities consume the available dissolved organic

matter to meet their C-demands (Fig. 4). Herbaceous plants assimilated greater relative amounts of NH_4^+ compared to woody plants, possibly due to differences in root architectural traits between these two growth forms. Still, all plants predominantly relied on NO_3^- made available through the fixation of atmospheric N_2 and organic N decomposition, followed by autotrophic nitrification (Fig. 4).

Acknowledgements

We are grateful to the Secretaría de Ambiente de Córdoba, especially to the Chancaní Reserve manager José González, for access to the study plots. We thank F. Aguirre, V. Da Silva, A. Devigili, L. D. Gorné, N. Graziani., J. Whitworth Hulse, M. Lanza, and M. L. Lipoma for support during field work, N. Cecchetto, N. Graziani, K. Van Nieuland and J. Vermeulen for assistance during analytical analyses and J. Corte for the provision of accommodation in Chancaní. This study is a contribution of Núcleo DiverSus endorsed by DIVERSITAS and Future Earth, and supported by FONCyT, CONICET, Universidad Nacional de Córdoba and the Inter-American Institute for Global Change Research (IAI) CRN 2015 and SGP-CRA2015 (which were supported by the National Science Foundation grants GEO-0452325 and GEO-1138881). D.H. is a postdoctoral fellow funded by the European Commission (International Outgoing Fellowships for career development (IOF) - Marie Curie Actions, PIOF-GA-2011-301443) and the Fund for Scientific Research - Flanders (FWO).

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743

744

745 **Table 1.** The 16 plant species and their family membership examined in this study along with their growth form and relative
746 abundance in the forest community.

Species	Family	Growth form	Abundance (%)
<i>Mimozyanthus carinatus</i>	Fabaceae	Woody	15.3
<i>Aspidosperma quebracho-blanco</i>	Apocynaceae	Woody	11.3
<i>Acacia furcatispina</i>	Fabaceae	Woody	8.8
<i>Larrea divaricata</i>	Zygophyllaceae	Woody	8.5
<i>Celtis erhembergiana</i>	Celtidaceae	Woody	8.0
<i>Tricomaria usillo</i>	Malpighiaceae	Woody	7.8
<i>Condalia microphylla</i>	Rhamnaceae	Woody	3.9
<i>Prosopis flexuosa</i>	Fabaceae	Woody	3.4
<i>Capparis atamisquea</i>	Capparaceae	Woody	2.6
<i>Geoffroea decorticans</i>	Fabaceae	Woody	1.2
<i>Bromelia urbaniana</i>	Bromeliaceae	Herbaceous	5.2
<i>Ephedra spp.</i>	Ephedraceae	Herbaceous	2.4
<i>Trichloris crinita</i>	Poaceae	Herbaceous	1.3
<i>Cordobia argentea</i>	Malpighiaceae	Herbaceous	1.0
<i>Gouinia paraguayensis</i>	Poaceae	Herbaceous	0.8
<i>Selaginella sellowii</i>	Selaginellaceae	Herbaceous	0.2

747

749 **Table 2.** Descriptive statistics of root traits of excavated herbaceous (H) and woody (W) species

	Minimum		Maximum		Mean		Median		Coefficient of variation	
	H	W	H	W	H	W	H	W	H	W
Specific root length (cm g ⁻¹)	59	8	12211	123	2238	33	260	26	2.18	1.02
Root tissue density (g cm ⁻³)	0.8	0.7	1.7	1.2	1.4	1.0	1.4	1.0	0.25	0.15
Median diameter (mm)	0.003	0.21	0.41	0.83	0.18	0.39	0.18	0.29	0.92	0.54
Mean diameter (mm)*	0.02	0.21	0.40	1.62	0.19	0.56	0.17	0.45	0.74	0.78
Branching intensity (# tips cm ⁻¹)	0.37	0.15	4.43	0.45	1.70	0.30	1.42	0.32	0.88	0.34
Root extinction coefficient β (-)	0.52	0.89	0.90	0.96	0.80	0.93	0.86	0.94	0.18	0.02
Fine root nitrogen (%)	0.67	1.29	2.17	4.28	1.30	2.26	1.07	1.82	0.45	0.48
Fine root carbon (%)	23.9	36.6	42.7	45.1	33.9	41.7	35.6	42.0	0.21	0.07
Mycorrhizal colonization (%)	0.0	0.0	38.2	51.8	23.2	15.5	26.5	8.8	0.72	1.14

*mean diameter of first and second terminal order roots

750 **Figure Legends**

751 **Fig. 1.** Standing pool concentrations of NO_3^- , NH_4^+ , and dissolved organic N (DON) and C:N ratios of dissolved organic matter
752 (DOC:DON) as a function of soil depth (values presented are averages of four replicate study plots; error bars indicate plus minus one
753 standard error)

754

755 **Fig. 2.** $\delta^{15}\text{N}$ values of whole plant biomass represented as a boxplot for all 16 species (a) and $\delta^{15}\text{N}$ values of NO_3^- , NH_4^+ , and dissolved
756 organic N (DON) (b) (error bars indicate plus minus one standard error; the value in brackets indicates the number of samples used to
757 calculate averages and standard errors; the $\delta^{15}\text{N}$ - NH_4^+ values were not determined for the 20-30 cm, 30-40 cm, and 40-60 cm soil layer
758 due to low concentration values)

759

760 **Fig. 3.** MixSIAR probability density functions representing the proportional contribution of DON, NH_4^+ and NO_3^- to overall N uptake
761 patterns for (a) woody and (b) herbaceous plants, and their associated matrix plots (c) and (d).

762

763 **Fig. 4.** Conceptual representation of the soil N cycle in the semi-arid study system, indicating the importance of 4 N transformation
764 pathways: (1) microbial N uptake of dissolved organic N, (2) microbial recycling of dissolved organic N, (3) free-living atmospheric
765 N_2 fixation, and (4) autotrophic nitrification. The thickness of the arrows represents the relative importance of the flux.

Fig. 1

Fig. 2

Fig. 3

Fig. 4