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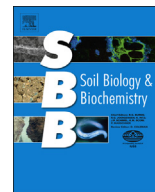
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Root exudate carbon mitigates nitrogen loss in a semi-arid soil



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ABSTRACT

The need for increased food production to support the growing global population requires more efficient nutrient management and prevention of nitrogen (N) losses from both applied fertiliser and organic matter (OM) decomposition. This is particularly important in semi-arid rainfed cropping soils, where soil water and temperature are the dominant drivers of N cycling rather than agricultural management. Here we used ¹⁴C and ¹⁵N techniques to examine how peptide/amino acid turnover, gross and net N transformation rates and nitrous oxide (N₂O) emissions responded to long-term plant residue additions and/or short-term root exudate additions. Soil was collected from a semi-arid rainfed field trial with one winter crop per year followed by a summer fallow period, where additional inputs of straw/chaff over 10 years had increased total soil organic C (SOC) by 76% compared to no extra additions (control). These field soils were incubated in the laboratory with or without a synthetic root exudate mixture at a range of temperatures reflecting regional field conditions (5–50 °C). Long-term plant residue additions (to build up total soil OM) did not decrease the risk of N loss as defined by the nitrification:immobilisation (N:I) ratio at most temperatures, so was not an effective management tool to control N losses. In comparison, short-term root exudate additions decreased the risk of N loss at all temperatures in both the control and plant residue treatment field soils. Increased net N mineralisation and decreased microbial C use efficiency at temperatures greater than 30 °C resulted in significant ammonium (NH₄⁺) accumulation. Microbial decomposers appeared to use amino acid-C for growth but peptide-C for energy production. Findings indicate that the greatest risk of N loss in these semi-arid soils will occur during rains at the start of the growing season, due to inorganic N accumulation over summer fallow when there are high soil temperatures, occasional significant rainfall events and no growing plants to release root exudates. While most attempts to manipulate the soil N cycle have occurred during the winter cropping period, our findings highlight the need to manage N supply during summer fallow if we are to minimise losses to the environment from semi-arid soils.

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1. Introduction

Concerns regarding food security, low fertiliser use efficiencies and the need to decrease greenhouse gas emissions necessitate the development of more sustainable agricultural systems. Semi-arid and arid regions cover approximately half of the global agricultural area (The World Bank, 2008) and are thus of major importance to food production and associated nutrient management. Sustainable agriculture in semi-arid regions presents unique challenges, especially in rainfed cropping systems, where rainfall and

temperature are the main drivers of microbial activity and cycling of nutrients such as nitrogen (N; Noy-Meir, 1973; Hoyle and Murphy, 2011). Semi-arid regions in the Southern Hemisphere have experienced a drying trend since the 1970s predominantly at the start of the grain-growing season (April and May; Cai et al., 2012). Although there has been a reported 15% decrease in heavy winter rainfall between 1950 and 2003 (Nicholls, 2010), summer rainfall events that occur outside of the period of crop and annual pasture growth are increasing (Alexander et al., 2007). More summer rainfall is expected to increase soil organic matter (OM) decomposition and N supply at a time when there is limited or no plant N uptake (Murphy et al., 1998; Austin et al., 2004). Nitrogen supply in excess of microbial demand results in N release, which is at risk of loss to the environment if nitrified.

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Nitrogen losses are undesirable, potentially limiting crop yield and having detrimental off-site environmental impacts [e.g. N leaching and emissions of nitrous oxide (N₂O)]. Management practices that mitigate N losses therefore need to be developed. Losses of N are particularly difficult to mitigate when they are not in response to N fertiliser additions, but originate from soil OM decomposition. The timing of inorganic N release from soil OM decomposition is difficult to change (Hoyle and Murphy, 2011) as peptide and amino acid turnover is primarily regulated by water and temperature (Jones et al., 2009; Farrell et al., 2013). Instead, management options are more likely to succeed when targeting the subsequent fate of the released inorganic N. Nitrification is the key pathway for N loss, as nitrate (NO₃⁻) is susceptible to leaching, and the greenhouse gas N₂O may be produced during and after nitrification (Wrage et al., 2001). One way to decrease potential N loss is by increasing microbial N immobilisation when plant N demand is low and thus decreasing the amount of inorganic N that is available for nitrification and loss (Crews and Peoples, 2005). The nitrification to immobilisation ratio, or *N:I* ratio, represents the balance between the N loss and retention pathways (Aber, 1992; Tietema and Wessel, 1992). This index has been correlated with NO₃⁻ leaching losses in temperate grassland and arable soils (Stockdale et al., 2002), but little is known about the behaviour of the *N:I* ratio in cropped soils from other climates.

Increased microbial N immobilisation and decreased potential for N loss could be achieved through manipulation of soil carbon (C) availability. Soil organic C (SOC) and N cycles are inextricably linked: both N mineralisation and immobilisation pathways are mediated by heterotrophic microorganisms, which require C from organic sources for growth and production of energy. When heterotrophic immobilisers are limited by C compared to N, net production of inorganic N occurs, which is then at risk of nitrification and subsequent loss (Barraclough, 1997). In contrast, organic sources with high C:N ratios can stimulate immobilisation. The majority of our understanding about soil C and N cycling processes has been gained through research in temperate and humid environments, but N cycling appears to behave unexpectedly in semi-arid regions, particularly in warm and dry seasons (Parker and Schimel, 2011; Sullivan et al., 2012). For example, in Californian grassland soil, net N mineralisation rates and nitrification potentials are greater during the warm dry summer than during the cooler, wetter winter, resulting in greater NH₄⁺ pools in the summer (Parker and Schimel, 2011). In addition, in south-west Australian soils, heterotrophic N immobilisation becomes constrained at temperatures greater than 30 °C, and mineralisation–immobilisation turnover becomes decoupled, resulting in the accumulation of inorganic N (Hoyle et al., 2006; Luxhøi et al., 2008). This mineralisation–immobilisation turnover decoupling at elevated temperatures was hypothesised to be due to C substrate limitation, caused by microorganisms consuming available C faster than C could be replaced by diffusion from nearby soil microsites (Hoyle et al., 2006). We hypothesised therefore that increasing soil C availability will decrease the potential of N loss (i.e. decrease the *N:I* ratio), especially at elevated temperatures that occur during summer in some semi-arid soils.

Soil organic C content and availability may be increased by agricultural management practices. These practices include plant residue inputs over the longer term (Dick, 1992; Liu et al., 2014), or shorter term rhizosphere processes such as inputs of labile C from root exudates and mycorrhiza (Jones et al., 2004; Kaiser et al., 2014). The objective of this research was to understand how different sources of C alter N transformations in arable semi-arid soil. Specifically, we investigated how long-term soil amendment with plant residues and/or short-term root exudate additions affected (a) N decomposition pathways; and (b) the subsequent fate of N and risk

of N loss as defined by the *N:I* ratio, under conditions reflective of both summer and winter temperatures in semi-arid soils.

2. Methods

2.1. Study site and field soil collection

Soil was collected from a field research site approximately 221 km north-northeast of Perth in the agricultural production zone (wheatbelt) of Western Australia (30.00° S, 116.33° E). The soil is a sand (92% sand, 2% silt, 6% clay) and classified as a Basic Regolithic Yellow-Orthic Tenosol (Australian soil classification; Isbell, 2002), or a Haplic Arenosol (IUSS Working Group WRB, 2007). The area has a semi-arid climate with cool, wet winters and hot, dry summers (Fig. 1). At the weather monitoring station closest to the study site (Dalwallinu, 30.28° S, 116.67° E) the historical mean annual rainfall is 288 mm and mean monthly temperatures range from 5.8 to 35.3 °C (1997–2013 data; Commonwealth of Australia Bureau of Meteorology, <http://www.bom.gov.au/climate/data>). Soil temperatures at 5 cm depth at the research site ranged from 6.2 to 45.6 °C (2008–2012; measured using a CS Model 107 Temperature Probe, Campbell Scientific, Logan, Utah, USA).

The field site consisted of two plant residue treatments: (i) import of additional plant residues to the soil (i.e. build-up of total soil OM; defined as '+OM') and (ii) control soil (i.e. no additional plant residue inputs; defined as 'No OM'). The +OM addition consisted of 20 t ha⁻¹ of barley straw, canola chaff and oaten chaff in 2003, 2006 and 2010 respectively. This represented an additional input to soil of 27 t C ha⁻¹, of which 7.9 t C ha⁻¹ was retained as SOC (i.e. microbial C use efficiency of 29%). This equated to 76% more SOC and 57% more total N in +OM soil than in No OM control soil (Table 1). All field plots were tilled using offset disks before seeding to 10 cm depth and seeded with knife point tines to 10 cm depth. Treatment plots (80 m by 10 m) were randomly allocated to three replicate blocks when the experimental site was established (2003), and have since been planted to an annual crop each winter (lupin-wheat-wheat rotation).

Soil was collected (Ap horizon; 0–10 cm) from each of the three replicate field treatments in May 2011 while the soil was dry (0.012 g H₂O g⁻¹ dry soil) and before winter rain commenced. A composite soil sample of 18 cores (7 cm diameter, 10 cm depth) was collected from each treatment plot in a zigzag sampling pattern, sieved (<2 mm) and stored at 4 °C until further analysis. Each field replicate (*n* = 3) was kept separate for use in the laboratory experimental design.

2.2. Laboratory experimental design

To investigate how long-term plant residue inputs and/or short-term root exudate additions affected soil N cycling at different temperatures, soils collected from the two field treatments (+OM and No OM; as described above) were incubated with (+RE) or without (No RE) synthetic root exudates at four or seven temperatures depending on soil process (details below), with three replicates per field treatment. Low molecular weight organic matter (LMWOM) turnover was investigated at four soil incubation temperatures (5, 15, 30 and 50 °C), while the other N transformation rate measurements were investigated at seven temperatures (5, 10, 15, 20, 30, 40 and 50 °C). Laboratory experimental conditions reflected soil temperatures ranging from winter cropping (5 °C) to summer fallow (50 °C). Synthetic root exudates were used to simulate conditions when plants are present in the soil. The root exudate solution consisted of D-glucose (6.75 mM); D-fructose and D-sucrose (1.35 mM each); succinic acid, citric acid, L-malic acid and

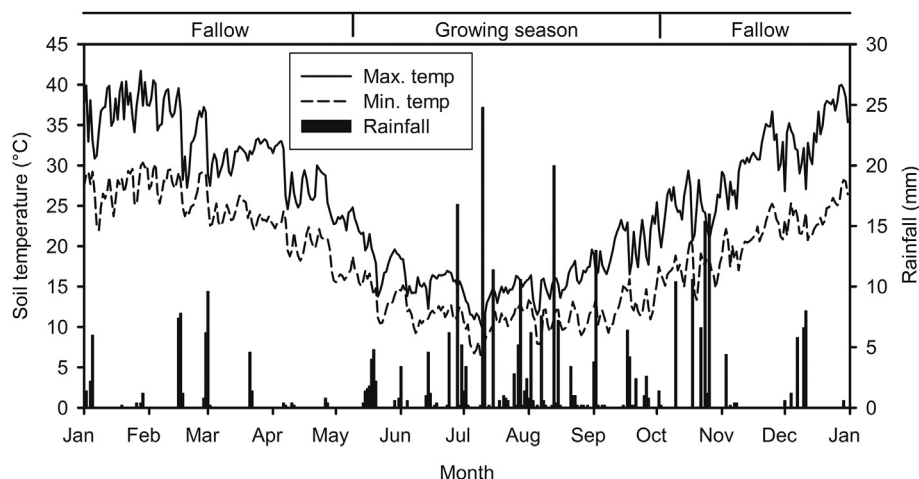


Fig. 1. Daily maximum and minimum soil temperatures at 5 cm depth and daily rainfall for 2011 at the research site.

Table 1

Properties of field trial soils (0–10 cm depth) collected 8 years after organic matter treatments were imposed. Values represent means \pm standard errors of the mean (SEM) ($n = 3$). Abbreviations: OM, organic matter; LFOM, light fraction organic matter; DOC, dissolved organic carbon; MBC, microbial biomass carbon.

	No OM soil	+OM soil
Soil pH _{CaCl₂} ^a	6.17 \pm 0.19	6.30 \pm 0.11
Total carbon (%) ^b	0.76 \pm 0.08	1.36 \pm 0.21 ^c
Total nitrogen (%) ^b	0.07 \pm 0.00	0.11 \pm 0.02
Soil C:N ratio	11.2 \pm 0.6	12.3 \pm 0.3
LFOM carbon (mg C g ⁻¹) ^{b,d}	0.91 \pm 0.13	2.04 \pm 0.39
LFOM nitrogen (mg N g ⁻¹) ^{b,d}	0.05 \pm 0.01	0.12 \pm 0.03
LFOM C:N ratio	17.0 \pm 0.23	16.6 \pm 0.45
DOC (μ g C g ⁻¹) ^e	120.4 \pm 3.8	236.0 \pm 26.3 ^c
MBC (μ g C g ⁻¹) ^f	118.4 \pm 17.3	218.9 \pm 43.7

^a Soil pH measured in 0.01 M CaCl₂ with a 1:5 soil:extract ratio.

^b Total C, total N, LFOM C and LFOM N determined by dry combustion of finely ground air-dry soil or LFOM using an Elementar Vario MACRO CNS Elemental Analyzer (Hanau, Germany).

^c +OM soil significantly different from No OM soil at $P < 0.05$.

^d Light fraction organic matter recovered by density separation with deionised water (1.05 g cm⁻³).

^e Dissolved organic C was extracted using 0.5 M K₂SO₄ (20 g soil to 80 mL extract) and analysed using an OI Analytical Aurora 1030 Wet Oxidation TOC Analyzer (College Station, TX, USA).

^f Microbial biomass C determined by fumigation–extraction (Brookes et al., 1985), analysed for oxidisable C as described for DOC, and then a k_{EC} factor of 0.45 used to convert the oxidisable C ‘flush’ into MBC (Wu et al., 1990).

fumaric acid (675 μ M each); and glycine, L-leucine, L-alanine, L-valine, L-serine, L-glutamic acid, L-aspartic acid, L-lysine, L-arginine, and L-phenylalanine (135 μ M each). This solution delivered 50 μ g C g⁻¹ dry soil (C:N ratio of 38:1) in 250 μ L for LMWOM turnover (added to 5 g soil) or in 1 mL for all other N transformation measurements (added to 20 g soil).

Soil was pre-incubated for 7 d at the specified temperatures to pre-condition the soil microorganisms, as microbial community structure and function differ with incubation temperature (Cookson et al., 2007). On day 1 and day 4 (during pre-incubation) and on day 8 (coinciding with ¹⁴C or ¹⁵N application; see sections below) the synthetic root exudate mixture, or an equivalent amount of deionised water (≤ 18.2 M Ω), was added to the soil. In total the +RE treatment received 100 μ g C g⁻¹ dry soil and 2.64 μ g N g⁻¹ dry soil prior to measurement of LMWOM turnover and N transformation rates. This was equivalent to the mass of C contained in the microbial biomass of the No OM soil (Table 1) and was thus sufficient to ensure no C limitation to the microbial population during incubation.

2.3. Peptide and amino acid turnover

Mineralisation of LMWOM substrates was examined by determining ¹⁴C-labelled peptide and amino acid turnover. Five grams of soil was placed in 50 mL polypropylene vials and pre-incubated for 7 d, with 125 μ L of either synthetic root exudate mixture or deionised water added to the soil on days 1 and 4 as described above. To determine the rate of ¹⁴CO₂ evolution after pre-incubation, 250 μ L of the synthetic root exudate mixture (another 50 μ g C g⁻¹ dry soil and 1.32 μ g N g⁻¹ dry soil) or deionised water was spiked with either ¹⁴C-labelled peptide (L-trialanine, ~1.5 mM, 0.28 kBq, 0.008 μ Ci, American Radiochemicals Inc., USA) or ¹⁴C-labelled amino acids (~3 mM, 0.27 kBq, 0.007 μ Ci, American Radiochemicals Inc., USA) and added to the surface of the soil on day 8. The amino acids were an equimolar mixture of 0.2 mM ¹⁴C-labelled L-amino acids (alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine, valine).

To capture evolved ¹⁴CO₂, a 1 M NaOH (1 mL) trap was placed inside each polypropylene vial and suspended above the soil and the vial hermetically sealed. ¹⁴CO₂ evolution was monitored by replacing the NaOH trap after 0.5, 1, 2, 4, 8, 10, 24, 48, 120, 144 and 168 h. The ¹⁴C content of the 1 M NaOH traps was determined by adding Scintisafe 3[®] scintillation cocktail (Fisher Scientific, Loughborough, UK) and the ¹⁴C content was subsequently measured using a Wallac 1404 liquid scintillation counter (Wallac EG&G, Milton Keynes, UK).

2.4. Modelling ¹⁴C dynamics

Mineralisation of LMWOM was modelled from the amount of ¹⁴C substrate remaining in the soil using SigmaPlot 12.3 (Systat Software Inc., San Jose, CA) and confirmed with R version 3.1.0 (R Foundation for Statistical Computing, Vienna, Austria). For the majority of treatments a double first-order exponential decay model was fitted to the data to represent a biphasic pattern of mineralisation:

$$f = (a_1 \times \exp^{-k_1 t}) + (a_2 \times \exp^{-k_2 t}) \quad (1)$$

where f is the amount of ¹⁴C remaining in the soil, a_1 and a_2 describe the size of the each mineralisation pool, k_1 and k_2 correspond to the respective rate constants for each mineralisation phase, and t is time. The first rapid phase described by k_1 is

considered to reflect $^{14}\text{CO}_2$ efflux as substrates are immediately used for catabolic processes (i.e. respiration; Boddy et al., 2007). The remaining ^{14}C -substrate is considered to be immobilised in the microbial biomass via anabolic processes. The second, slower mineralisation phase (k_2) is attributed to the use of this C temporarily immobilised in the biomass (i.e. storage-C; Boddy et al., 2007; Farrar et al., 2012).

For some treatments at 5 and 50 °C a simpler first-order exponential model with asymptote fitted the data better:

$$f = y_0 + (a \times \exp^{-kt}) \quad (2)$$

where the asymptote value y_0 describes a pool that is unavailable for microbial mineralisation (recalcitrant C), a describes the size of a single, very slowly mineralisable pool (labile C) with k representing the exponential decay constant for this pool, and t is time.

The substrate half-life for the first mineralisation pool (a_1 and a for double and single exponential decay models respectively) was calculated using the following equation:

$$t_{1/2} = \ln(2)/k_1 \quad (3)$$

To calculate the proportion of C immobilised by the microbial biomass, we calculated the microbial C use efficiency for double exponential decay data as follows (Manzoni et al., 2012; Sinsabaugh et al., 2013):

$$\text{Mic}_{\text{CUE}} = a_2/(a_2 + a_1) \quad (4)$$

where a_2 is the amount of C immobilised by the microbial biomass (i.e. difference between total ^{14}C input minus $^{14}\text{CO}_2$ evolved), and a_1 accounts for C lost through respiration ($^{14}\text{CO}_2$ evolved).

For data where a single exponential decay equation was used, the following equation was used.

$$\text{Mic}_{\text{CUE}} = y_0/(y_0 + a_1) \quad (5)$$

where y_0 is the amount of recalcitrant C and a_1 accounts for C lost through respiration ($^{14}\text{CO}_2$ evolved). Since the amount of C immobilised by the microbial biomass was determined by difference (i.e. total ^{14}C input minus $^{14}\text{CO}_2$ evolved) this could include substrate in the microbial biomass as well as any ^{14}C substrate remaining in the soil at the end of the incubation period. However, residual ^{14}C substrate is considered to be negligible as ^{14}C substrates such as amino acids and oligopeptides are rapidly immobilised by the microbial biomass leaving less than a few percent of the total in solution after a few hours (Farrell et al., 2013). In addition, transfer of ^{14}C to humified soil OM would be in significant over the time courses represented in these experiments.

2.5. Gross N transformation rates and inorganic N

Gross N transformation rates were determined by ^{15}N isotopic pool dilution (Kirkham and Bartholomew, 1954). The bulk soil samples were adjusted to 45% water filled pore space (WFPS), mixed, left to equilibrate overnight at 4 °C, and then 20 g of soil was packed into 120 mL vials to a bulk density of 1.4 g cm⁻³. Subsamples of each treatment combination were then pre-incubated for 7 d at temperatures representative of field soil conditions (5, 10, 15, 20, 30, 40 and 50 °C). Each vial was placed inside an airtight glass jar fitted with gas septum ports in the lids to enable headspace gas analysis. Water (10 mL) was added to the jars (but not in contact with the soil) to maintain humidity and thus minimise soil drying. The jars were vented every 24 h to maintain an aerobic environment. On days 1 and 4, 0.5 mL of either synthetic root exudate

solution or deionised water (≤ 18.2 M Ω) was added to subsample vials, for a total of 50 $\mu\text{g C g}^{-1}$ dry soil and 1.32 $\mu\text{g N g}^{-1}$ dry soil during pre-incubation.

After pre-incubation, 1 mL of ^{15}N -enriched (60 atom %) ammonium sulphate [$(^{15}\text{NH}_4)_2\text{SO}_4$] was applied as multiple droplets to each vial of soil to obtain a concentration of 5 $\mu\text{g N g}^{-1}$ dry soil. The +RE treatment also received a second application of synthetic root exudates (50 $\mu\text{g C g}^{-1}$ dry soil and 1.32 $\mu\text{g N g}^{-1}$ dry soil) in the same 1 mL aliquot as the $(^{15}\text{NH}_4)_2\text{SO}_4$. The 1 mL aliquot increased the soil water content to approximately 60% WFPS: below the WFPS at which nitrification becomes limited by oxygen exchange in soils from this study region (Gleeson et al., 2010), and at the upper limit of soil WFPS observed in the field (Barton et al., 2011, 2013). The vials were incubated inside airtight jars at the temperatures described above until they were extracted. Two extraction times were selected based on a previous ^{15}N isotopic pool dilution study by Hoyle et al. (2006) in a similar semi-arid soil: $T_0 = 4\text{--}6$ h and $T_1 = 24$ h after ^{15}N addition. At each of these times, subsamples were shaken with 80 mL of 0.5 M K_2SO_4 for 30 min then filtered through Whatman No. 42 filter paper using Buchner funnels under vacuum. The extracts were kept frozen until further analysis for inorganic N concentration and ^{15}N enrichment.

Ammonium and NO_3^- concentrations in soil extracts were determined by colourimetric analysis using the modified Berthelot reaction for NH_4^+ (Krom, 1980; Searle, 1984) and the hydrazinium reduction method for NO_3^- (Kamphake et al., 1967; Kempers and Luft, 1988) on a Skalar San Plus auto-analyser (Skalar Inc., Breda, The Netherlands). Net N mineralisation and net nitrification rates over the 7 d pre-incubation were calculated from the difference between NH_4^+ and NO_3^- concentrations before and after pre-incubation.

The soil extracts were prepared for $^{15}\text{N}/^{14}\text{N}$ isotope ratio analysis using a modified diffusion method (Brooks et al., 1989; Sørensen and Jensen, 1991). The NH_4^+ and NO_3^- within the extracts was, in a two-stage process, trapped on separate acidified diffusion disks as ammonia. The disks were subsequently analysed for %N and $^{15}\text{N}/^{14}\text{N}$ isotope ratio by the UC Davis Stable Isotope Facility, using an Elementar Vario EL Cube Elemental Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 IRMS (Sercon, Cheshire, UK). For further details see the UC Davis website (<http://stableisotopefacility.ucdavis.edu/>).

Any residual inorganic N remaining in the soil after extraction was removed by filtering with a further 80 mL of 0.5 M K_2SO_4 and then two subsequent 80 mL volumes of deionised water. The washed soil was dried at 70 °C, ground to a fine powder, and analysed for %N and $^{15}\text{N}/^{14}\text{N}$ isotope ratio as described above. Total recovery of applied ^{15}N after 24 h from the NH_4^+ , NO_3^- and residual soil pools was on average 98% (data not shown).

2.6. Modelling N transformation rates

The numerical model FLUAZ (Mary et al., 1998) was used to simulate gross N transformation rates. The lowest mean weighted errors were obtained when mineralisation and nitrification were modelled using zero-order kinetics, immobilisation using first-order kinetics and we assumed no denitrification. Denitrification was set to zero in the model as measured N_2O fluxes were low (see Results section 3.3) and a sensitivity analysis indicated no influence of these low N_2O fluxes on modelled gross N transformation rates. The model was unable to simulate gross N cycling rates at 40 and 50 °C in some of the soils due to a convergence problem between the fitted parameters, which may be linked to observed rapid dilution of ^{15}N enrichment of the NH_4^+ pool prior to T_0 at elevated temperatures.

2.7. Nitrous oxide analysis

Nitrous oxide fluxes were determined by collecting headspace gas samples (15 mL) from all treatment jars 24 h after ^{15}N was added (i.e. before soil extraction). Three replicate samples of the background concentration of N_2O in air were also taken prior to closing the jars. Samples were stored in 12 mL Labco Exetainers under positive pressure before analysis for concentration and ^{15}N atom % of N_2O by the UC Davis Stable Isotope Facility using a ThermoFinnigan GasBench + PreCon trace gas concentration system interfaced to a ThermoScientific Delta V Plus IRMS (Bremen, Germany). For further details see the UC Davis website (<http://stableisotopefacility.ucdavis.edu/>).

2.8. Statistical analysis

Analysis of variance (ANOVA) with associated TukeyHSD post hoc tests were performed using R version 3.1.0 (R Foundation for Statistical Computing, Vienna, Austria) to determine if there were significant differences between the soil properties of the treatments. A mixed model, PROC MIXED from SAS version 9 (SAS Institute Inc., Cary, NC, USA) was used to determine significant effects of addition of plant residue inputs, addition of synthetic root exudates and temperature on ^{14}C -labelled LMWOM substrate half-lives, microbial C use efficiencies, inorganic N, net N cycling rates, N_2O flux and $^{15}\text{N}_2\text{O}$ enrichment. Gross N transformation rates were compared using the 95% confidence intervals generated by the FLUAZ model.

3. Results

3.1. Soil organic matter turnover (N supply)

Peptide mineralisation data was best represented by a double first-order exponential decay model ($r^2 = 0.9904 \pm 0.0024$), except for soil incubated at 50 °C and +RE soil incubated at 5 °C. A single-order exponential decay model with asymptote ($r^2 = 0.9733 \pm 0.0052$) was fitted to the exceptions. Amino acid mineralisation data was also best described by a double first-order exponential decay model ($r^2 = 0.9974 \pm 0.0003$).

Long-term additional plant residue inputs (+OM) had no significant effect on the microbial C use efficiencies of peptides (mean of 42.8% at 5–30 °C) or amino acids (mean of 70.8% at 5–30 °C) except at 50 °C where +OM soil was greater than No OM soil ($P < 0.0001$; Fig. 2). Short-term addition of synthetic root exudates (+RE) decreased peptide C use efficiencies at 5 and 50 °C by a mean of 14% ($P < 0.05$), but increased peptide C use efficiencies at 30 °C by a mean of 20% ($P < 0.0001$; Fig. 2a,b). Root exudates also decreased amino acid C use efficiencies at 50 °C by a mean of 14% ($P < 0.0001$), but not at other temperatures ($P > 0.05$; Fig. 2c,d). Across all treatments, C use efficiencies were about half at 50 °C compared to 30 °C ($P < 0.0001$).

Both plant residue (+OM) and root exudate (+RE) inputs to soil had minimal effect on peptide and amino acid turnover (i.e. half-lives) between 15 and 30 °C (Fig. S1a–d). However at 5 °C, the half-lives of both peptides and amino acids significantly decreased in the +OM treatment ($P < 0.05$; Fig. S1a–d). Addition of synthetic root exudates (+RE) only increased amino acid half-lives at 5 °C ($P < 0.01$; Fig. S1c–d).

3.2. Nitrogen transformation rates and inorganic N pools

Gross N transformation rates increased linearly to 30 °C, at which point gross N mineralisation, nitrification and immobilisation rates averaged across both root exudate treatments (i.e.

mean of +RE and No RE) were 2-, 2.2- and 2.8-fold greater where plant residues were added (+OM) compared to the control field soil (No OM), respectively (Figs. 3a,b and 4a–d). In addition, net N mineralisation rates showed a significant increase from 30 to 50 °C; +OM soil (maximum $6.3 \mu\text{g N g}^{-1} \text{d}^{-1}$) was greater than No OM soil (maximum $2.5 \mu\text{g N g}^{-1} \text{d}^{-1}$; $P < 0.001$; Fig. 3c,d). Net N mineralisation rates were negligible from 5 to 20 °C ($0.02 \mu\text{g N g}^{-1} \text{d}^{-1}$) with no treatment effect (Fig. 3c,d). Microbial demand for NH_4^+ by nitrifiers (Fig. 4a,b) and immobilisers (Fig. 4c,d) decreased above 30 °C. As a consequence the NH_4^+ -N pool size rapidly increased from ca. $3 \mu\text{g N g}^{-1}$ at 30 °C to $60.5 \mu\text{g N g}^{-1}$ in the presence of additional plant residues (+OM soil) and to $24.9 \mu\text{g N g}^{-1}$ in No OM soil at 50 °C (+OM and No OM soils were significantly different at $P < 0.001$; Fig. 5a).

Addition of synthetic root exudates (+RE) caused a small increase in gross N mineralisation ($P < 0.05$; Fig. 3a,b), but had no effect on net N mineralisation ($P = 0.06$; Fig. 3c,d). Addition of synthetic root exudates caused a small decrease in gross nitrification at some temperatures in both plant residue field treatments (i.e. No OM and +OM soils; $P < 0.05$; Fig. 4a,b). In contrast root exudates caused on average a 3.9-fold increase in gross N immobilisation from 5 to 30 °C in both plant residue field treatments ($P < 0.05$; Fig. 4c,d).

Addition of synthetic root exudates (+RE) slightly but significantly decreased NH_4^+ pool size by a mean of $1.2 \mu\text{g N g}^{-1}$ ($P < 0.0001$; data not shown). Nitrate pool size in soil from the long-term plant residue treatment (+OM) soil was approximately twice as large at all temperatures compared to the control soil (No OM; $P < 0.0001$; Fig. 5b). Addition of synthetic root exudates had no effect on NO_3^- pool size ($P = 0.22$).

Net nitrification in the +OM soil was only greater than No OM soil at 30 °C ($P < 0.0001$; data not shown). Addition of synthetic root exudates had no effect on net nitrification rates ($P = 0.18$). Net nitrification rates increased with increasing temperature to a maximum at 30–40 °C then decreased substantially at 50 °C ($P < 0.001$).

3.3. Fate of inorganic N

Addition of synthetic root exudates (+RE) decreased the $N:I$ ratio to a greater extent and at a wider range of temperatures than increased plant residue inputs (+OM; Fig. 4e,f). Addition of synthetic root exudates significantly decreased the $N:I$ ratio over the temperature range 5–30 °C for the control field soil (No OM) and between 10 and 30 °C for the increased plant residue soil (+OM; $P < 0.05$). The $N:I$ ratio was < 1 in the presence of synthetic root exudates (+RE) at all temperatures (i.e. gross N immobilisation was greater than gross nitrification; Fig. 4f). In contrast, the $N:I$ ratio was > 1 for treatments from 10 to 30 °C without synthetic root exudates (Fig. 4e). Increased plant residue input (+OM) in soil without synthetic root exudates (No RE) decreased the $N:I$ ratio at temperatures from 10 to 30 °C ($P < 0.05$; Fig. 4e). However, the +OM treatment in the presence of root exudates (+RE) slightly increased the $N:I$ ratio but only at 15 °C ($P < 0.05$; Fig. 4f).

Nitrous oxide fluxes after the 7 d incubation period were low with maximum N_2O flux at 40 °C (mean of $0.005 \mu\text{g N g}^{-1} \text{d}^{-1}$; Fig. S2a). Long-term plant residue inputs (+OM) and addition of synthetic root exudates (+RE) had no effect on N_2O flux ($P = 0.13$; Fig. S2a). The ^{15}N enrichment range of N_2O (0.3–4.9 atom %; Fig. S2b) was the same as the ^{15}N enrichment range of the NO_3^- pool 24 h after ^{15}N addition (0.4–4.3 atom %) but lower than that of the NH_4^+ pool (3.0–54.2 atom %); this suggests that denitrification was the source of N_2O .

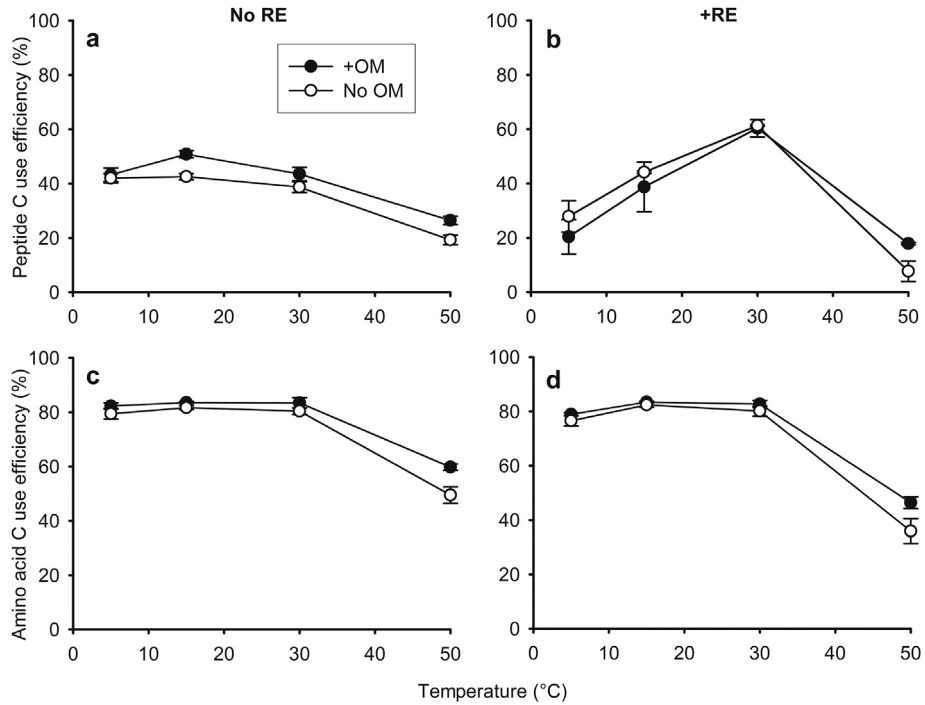


Fig. 2. Influence of temperature on microbial carbon use efficiencies of (a) ¹⁴C-labelled peptides without root exudates; (b) ¹⁴C-labelled peptides with root exudates; (c) ¹⁴C-labelled amino acids without root exudates; and (d) ¹⁴C-labelled amino acids with root exudates. Error bars are ±standard error of the mean (SEM) (n = 3), and may be smaller than the symbols. Legend is the same for all panels. Legend abbreviation: OM, organic matter.

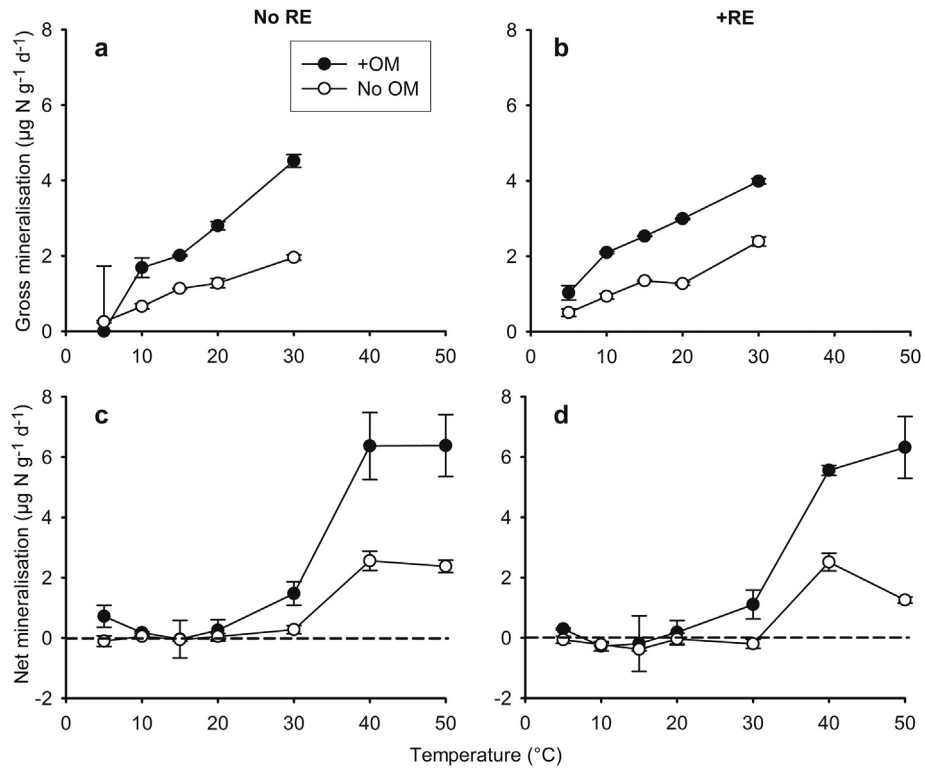


Fig. 3. Influence of temperature after 7 d of incubation on (a) gross N mineralisation without root exudates; (b) gross N mineralisation with root exudates; and influence of temperature over 7 d of incubation on (c) net N mineralisation without root exudates and (d) net N mineralisation with root exudates. Error bars for gross N mineralisation are 95% confidence intervals derived from the FLUAZ model, and for net N mineralisation are ±SEM (n = 3), and may be smaller than the symbols. Legend is the same for all panels. Legend abbreviation: OM, organic matter.

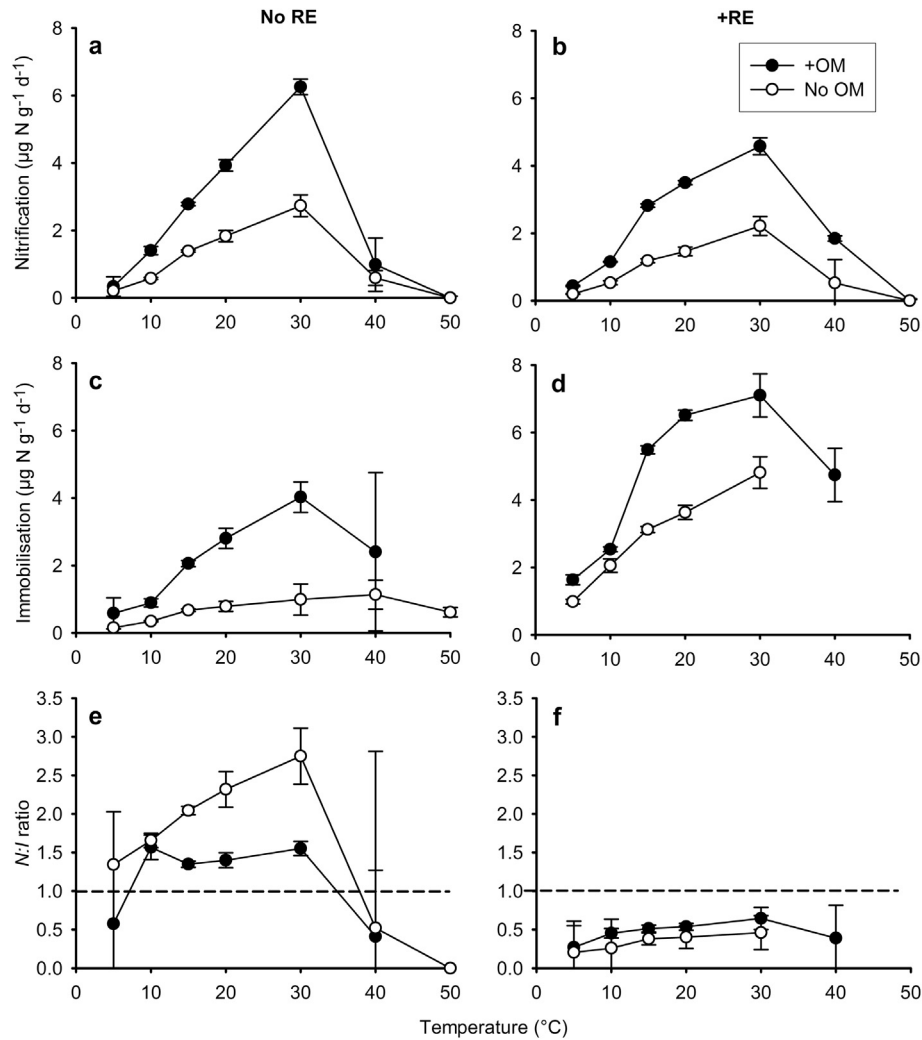


Fig. 4. Influence of temperature on (a) gross nitrification without root exudates; (b) gross nitrification with root exudates; (c) gross N immobilisation without root exudates; (d) gross N immobilisation with root exudates; (e) N:I ratio without root exudates; and (f) N:I ratio with root exudates. The dashed line at 1.0 in (e) and (f) represents the N:I ratio at which nitrification and N immobilisation rates are equal. Error bars are 95% confidence intervals derived from the FLUAZ model, and may be smaller than the symbols. Legend is the same for all panels. Legend abbreviation: OM, organic matter.

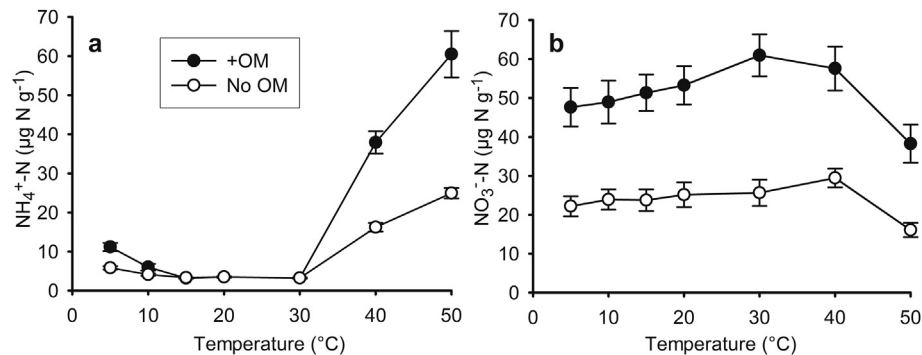


Fig. 5. Influence of temperature after 7 d of incubation on (a) $\text{NH}_4^+\text{-N}$; and (b) $\text{NO}_3^-\text{-N}$. Synthetic root exudate treatments were combined in the figure presented, as root exudates had no effect on $\text{NO}_3^-\text{-N}$ ($P > 0.05$) and the effect of root exudates on $\text{NH}_4^+\text{-N}$ was small compared to the effects of organic matter treatment and temperature. Error bars are $\pm\text{SEM}$ ($n = 6$), and may be smaller than the symbols. Legend is the same for all panels. Legend abbreviation: OM, organic matter.

4. Discussion

4.1. Sources of SOC to decrease the risk of N loss

Inputs of labile C to soil as synthetic root exudates decreased the risk of N loss to a greater extent than long-term inputs of plant

residues that increased total soil OM. Root exudate C decreased the risk of N loss by stimulating microbial N immobilisation (on average by 3.9-fold), and by slightly decreasing nitrification. By contrast, addition of plant residues increased both immobilisation and nitrification (on average by 2.7- and 2.8-fold respectively), but had no effect on the ratio between these two competing pathways for

inorganic NH_4^+ . Nitrification is often the principal process controlling inorganic N consumption in semi-arid soils due to C limitation of the heterotrophic microbial population (Hoyle et al., 2006). Root exudates appeared to remove C limitation of microbial heterotrophs, allowing them to compete more successfully for NH_4^+ . The synthetic root exudates used in the present study had a higher C:N ratio (38:1) compared to the bulk soil OM (12:1), creating an environment that is C-available but N-limited; this is conducive to microbial immobilisation of N (Paterson, 2003). Our findings are consistent with others who have found that labile C additions from rhizodeposition stimulate microbial N immobilisation, thus retaining N in soil (Clarholm, 1985; Qian et al., 1997).

Addition of crop residues to soil increased organic N retention, inorganic N supply and N loss pathways but did not decrease the risk of N loss. Plant residue inputs increased total SOC, DOC and LFOM-C by 1.8-, 2.0- and 2.2-fold respectively over eight years of treatment. Both DOC and LFOM are indicators of available C in soil, as these soil OM fractions are transient, turnover rapidly and are microbial substrates (Janzen et al., 1992; Haynes, 2005). In addition, cereal crop residues typically have C:N ratios ranging from 40:1–150:1 (Smith and Peckenpaugh, 1986; Singh et al., 2004; Hoyle and Murphy, 2011) so addition of residues to soil should also create an N immobilising environment, observed in the long-term by increased soil N retention in the build up plant residue treatment (+OM soil; Table 1). The amount of available C released during crop residue decomposition cannot however be explained by residue C:N ratio; the complex chemical composition of residues provides less available C than rhizodeposits, which are dominated by microbially available low molecular weight solutes such as sugars and amino acids (Kumar and Goh, 2000; Farrar et al., 2003). Even though there was more DOC and LFOM in the soil with plant residue inputs (+OM), the C:N ratio and LFOM C:N ratio of the two soils did not vary (Table 1), and overall the risk of N loss was not decreased. Instead plant residue additions up-regulated the entire soil N cycle. This suggests that the greater total pool of soil OM from plant residue inputs did not solely increase C availability to heterotrophic microorganisms, but also increased N supply through mineralisation, and thus had little effect on the balance between subsequent NH_4^+ retention and loss pathways. Our results are consistent with other studies with long-term increases in soil OM due to plant residue inputs, which generally increase microbial biomass, C and N contents and nutrient cycling (reviewed in Kumar and Goh, 2000). This is however in contrast to short-term additions of plant residues to soil, after which net N immobilisation and decreased inorganic N pools are often observed (for example Janzen and Kucey, 1988; Geisseler et al., 2012). Increasing total soil OM through long-term additions of crop residues was not an effective management tool to decrease N losses in this semi-arid environment.

4.2. Inorganic N accumulates at high soil temperature

Considerable NH_4^+ accumulation at soil temperatures representative of summer conditions was a result of decreased microbial C use efficiency coinciding with increased net N mineralisation. This NH_4^+ build-up was consistent with mineralisation–immobilisation turnover decoupling observed by Hoyle et al. (2006) and Luxhøi et al. (2008) at temperatures greater than 30 °C in similar semi-arid soils, while greater net N mineralisation has also been observed during hot, dry summer months compared to the cooler growing season in a Californian semi-arid grassland ecosystem (Parker and Schimel, 2011). The two main pathways of NH_4^+ production in soil are either by direct assimilation of LMWOM molecules into microbial biomass and subsequent release of N that is not required (Jones et al., 2013), or by mineralisation of organic N to

NH_4^+ by extracellular enzymes (Geisseler et al., 2010). Which pathway is dominant in soil depends on regulation of microbial enzyme production for N uptake of LMWOM or NH_4^+ , which in turn is hypothesised to depend on the main forms and relative availabilities of N and C in soil (Geisseler et al., 2010). Our results suggest that at elevated temperatures both C and N from LMWOM are less able to be incorporated into microbial biomass, so more NH_4^+ is released into the soil mineral N pool, whether due to extracellular or intracellular breakdown of LMWOM.

The mechanism for decreased microbial C use efficiency at soil temperatures greater than 30 °C in this semi-arid soil is likely related to a shift in the balance between microbial growth and respiration compared to lower temperatures. Microbial respiration is inherently more sensitive to temperature increases than microbial growth (Steinweg et al., 2008; Allison et al., 2010; Wetterstedt and Ågren, 2011), due to the increasing physiological costs of respiration and heat survival mechanisms (Schimel et al., 2007). Therefore at elevated temperatures microbes respire a larger proportion of any C they assimilate compared to C allocated to growth. In addition, increased C cycling and microbial metabolism at elevated temperatures can deplete readily accessible labile substrates, requiring microorganisms to use substrates of lesser quality, and which also lowers microbial C use efficiency (Manzoni et al., 2012; Sinsabaugh et al., 2013). In the present study, C cycling appears to be tightly linked to N cycling, as decreased microbial C use efficiency and assimilation of C at elevated soil temperatures is associated with NH_4^+ release and thus decreased assimilation of N.

4.3. Differences between amino acid and peptide turnover

Measurement of peptide and amino acid turnover suggested that these LMWOM molecules were used in differing ways by microbial decomposers in this semi-arid soil. Peptides turned over more rapidly than amino acids, as has been previously observed in a range of microorganisms and climates (Matthews and Payne, 1980; Farrell et al., 2013). Interestingly, we also observed that in this semi-arid soil, peptide-C appeared to be utilised for production of energy (more C was allocated to respiration), while amino acid-C was utilised for building biomass. Peptides and amino acids are taken up actively (i.e. using energy) by separate transport systems (Payne and Smith, 1994). Peptides may be preferentially used for energy production over amino acids because active uptake of one peptide molecule requires less energy than if the constituent amino acids were taken up separately, and peptides provide more C and N per molecule than amino acids (Matthews and Payne, 1980; Farrell et al., 2013). On the other hand, amino acids can be directly used for biosynthesis of proteins, but peptides must be hydrolysed before their constituent amino acids can be used for protein synthesis or as sources of C and N after further breakdown (Anraku, 1980; Payne, 1980). If the patterns of N turnover of these substrates follow C turnover, peptides may be the primary source of inorganic N after catabolism and release of peptide-C through respiration. Amino acid-N may instead be immobilised into microbial biomass concurrently with amino acid-C.

4.4. Implications for semi-arid environments

Effective management practices to decrease the risk of N loss in semi-arid soils still need to be found. The greatest risk of N loss in this arable semi-arid soil likely occurs from inorganic N that accumulates due to soil OM decomposition over summer. These inorganic N pools are then at risk of loss by NO_3^- leaching during rain events that mark the beginning of the cooler growing season

(Anderson et al., 1998; Arregui and Quemada, 2006). Risk of N loss by N₂O emissions in this semi-arid soil is also likely greatest during summer fallow: N₂O emissions at constant soil water content in the present study were at a maximum between 30 and 40 °C, and up to half of annual N₂O emissions in the field can be in response to summer and autumn rainfall events (Mummey et al., 1997; Barton et al., 2008, 2013).

Risk of N loss from soil is low when N supply is synchronised with plant N demand both in timing and amount (Murphy et al., 2004), as observed in undisturbed ecosystems with tight internal N cycling (Avila et al., 2002; Perveen et al., 2014). Tools that decrease the risk of N loss may therefore work by controlling N supply, increasing plant N demand, or by immobilising excess inorganic N in microbial or plant biomass (Crews and Peoples, 2005). Control of N supply in these rainfed semi-arid agricultural soils is difficult, because the majority of inorganic N availability (up to 80% of plant N uptake in wheat) is derived from microbial decomposition of crop residues and soil OM (Angus, 2001; Fillery, 2001), and OM decomposition is more reliant on changes in soil water availability and temperature than agricultural management practices (Hoyle and Murphy, 2011). While organic N retention and microbial immobilisation were increased in the present study, N supply and nitrification were also increased. As such, increasing total soil OM through long-term plant residue inputs was not effective at decreasing the risk of N loss. In this environment, changing tillage practices from conventional till to minimal or no-till is also unlikely to have an effect on soil OM storage and thus risk of N loss, as no difference in total C has been observed after seven years between tillage treatments (Cookson et al., 2008). The present study however highlighted the importance of root exudates for increasing microbial N immobilisation. Mitigation of N loss in these semi-arid soils therefore could involve increasing the extent and duration of actively growing plant roots, especially during late summer and early autumn. This might be achieved by incorporation of perennials into the current annual cropping system (Crews and Peoples, 2005), or summer crops. Root growth may also be increased in the early growing season by managing soil constraints that restrict root growth (e.g. sub-soil acidity, compaction layers) and by selective breeding for traits such as increased root branching and early growth vigour, which also increases root NO₃⁻ capture (Lynch, 1995; Hoad et al., 2001; Dunbabin et al., 2003).

5. Conclusions

Our ability to manipulate N transformation rates and decrease the risk of N loss from semi-arid soils will depend greatly on the C source (which is associated with the presence or absence of root exudates), and the time of year (as high soil temperature can cause potential disconnect in mineralisation–immobilisation turnover). Root exudate C was more effective than long-term increased plant residue inputs at decreasing the risk of N loss, by increasing the potential for heterotrophic microbial immobilisation relative to nitrification. In contrast, addition of plant residues to soil increased both N supply and N loss pathways. Therefore the greatest risk of N loss in this arable semi-arid soil occurs from soil inorganic N that accumulates over summer. This accumulation is in response to high net N mineralisation rates after occasional rainfall events, coupled with low microbial C use efficiency at elevated soil temperatures, and limitations to active plant growth. Potential loss of this accumulated inorganic N is subsequently greatest following opening rains in autumn prior to crop establishment, when drainage begins and soil temperatures cool allowing nitrification to occur. However, management practices to mitigate this risk of N loss still need to be found.

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Appendix A. Supplementary data

Supplementary data related to this chapter can be found at <http://dx.doi.org/10.1016/j.soilbio.2015.06.011>.

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