



## Original article

## Microbial activity and biomass and N and P availability in a saline sandy loam amended with inorganic N and lupin residues

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## ABSTRACT

Plant residue can be a cost effective source of N and P fertilisers, which may enhance plant growth in saline soils. Salinity and limited availability of N may limit microbial activity and thus residue decomposition N and P availability. A laboratory experiment was conducted to investigate the effects of  $\text{NH}_4\text{-N}$  or  $\text{NO}_3\text{-N}$  on microbial activity and biomass and N and P availability in a saline sandy loam. Three levels of salinity ( $\text{EC}_{1:5}$  0.21, 0.51 and  $0.85 \text{ dS m}^{-1}$ ) were imposed in the sandy loam using solutions of  $\text{Na}^+$  and  $\text{Ca}^{2+}$ . Soil was amended with or without 2% (w/w) lupin residues (C/N ratio 15.4) or  $50 \mu\text{g N g}^{-1}$  soil as  $\text{KNO}_3$  or  $(\text{NH}_4)_2\text{SO}_4$ . With no residue or inorganic N added, the concentration of available N and P remained unchanged over 45 days. Soil respiration and microbial biomass C, N and P decreased with increasing salinity, but significantly increased with residue addition. Addition of inorganic N had no significant effect, but addition of  $\text{NO}_3\text{-N}$  with residue significantly increased soil respiration and microbial biomass C, N and P. Salinity had no effect on N availability and decreased P availability. Nitrogen availability was lower with addition of  $\text{NH}_4\text{-N}$ , N than with  $\text{NO}_3\text{-N}$ . Available N and P increased with residue addition and increased further with addition of  $\text{NO}_3\text{-N}$  than with  $\text{NH}_4\text{-N}$ . The greater C availability in the lupin residue amended saline sandy loam stimulated microbial activity and biomass with greater N demand, thus promoted immobilization of  $\text{NO}_3$ . Hence, N and P availability increased in the saline sandy loam.

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## 1. Introduction

In saline soils, osmotic stress, coupled with low nutrient availability, limits plant growth. It has been shown that in saline soils plant growth can be improved by addition of inorganic N [1] and/or P [2]. Plant residues can be a cost effective source of N and P. Soil microorganisms play a key role in decomposition of plant residues and the release and consequently the availability of N and P to the plants [3]. Salinity has been widely reported to have a negative effect on microbial and enzyme activities [4] and microbial biomass [5].

It has been shown that addition of inorganic N had positive effects [6,7], negative effects [8,9] and sometimes no effects [10,11] on the microbial activity or the decomposition of plant residues. The preferential microbial uptake of ammonium rather than nitrate has been noted by Conde et al. [12] and Recous and Mary [13] and

was mostly attributed to the priming effect of  $\text{NH}_4\text{-N}$  on microbial activity.

In naturally saline-alkaline soils [14], or saline sodic soils [15], addition of easily degradable residues enhanced microbial immobilization of inorganic  $\text{NH}_4\text{-N}$  and increased the microbial biomass and soil respiration. Dendooven et al. [14] found assimilation of  $\text{NO}_3\text{-N}$  in alkaline saline soils with salinity ( $\text{EC}_e$ ; salinity at saturation in the soil paste) 56.0 and  $11.6 \text{ dS m}^{-1}$ . However, Azam and Ifzal [4] reported results indicating a higher sensitivity to NaCl of  $\text{NO}_3^-$  compared to  $\text{NH}_4^+$ -assimilating microorganisms.

The interactive effect of salinity with alkalinity can affect microbial response to N addition or form and decomposition of plant residues and subsequent release of nutrients. In saline soils, with increasing soil pH, microbial activity may be stimulated as alkali can dissolve, disperse or cause chemical hydrolysis of the added as well as the native organic matter [16]. In sodic soils, relative to  $\text{Ca}^{2+}$  ions, increased  $\text{Na}^+$  adsorption on clay minerals led to dispersion of clay particles and reduced soil aggregation thereby increasing organic matter decomposition [17]. Less is known about the actual rates of short-term microbial N and P transformations in the saline non-alkaline and non-sodic systems that differ in C availability and N availability. This study investigates the effects of

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inorganic N added as  $\text{NH}_4\text{-N}$  or  $\text{NO}_3\text{-N}$  on microbial activity and biomass and N and P availability in a lupin-amended saline sandy loam soil with pH at neutral.

## 2. Materials and methods

### 2.1. Soil characteristics

The study was conducted in a sandy loam soil collected from Monarto, South Australia (latitude  $35^\circ 05' \text{S}$ , longitude  $139^\circ 06' \text{E}$  and elevation 166 m). The soil is alluvium red duplex classified as red chromosol [18]. The characteristics of the soils are presented in Table 1. Several sub-samples (0–15 cm depth), were bulked to give a composite sample. The soils were air-dried and sieved to  $\leq 2$  mm. Soil pH and electrical conductivity (EC) were measured in 1:5 soil-water suspension after 1 h end-over-end shaking at  $25^\circ \text{C}$ . Soil bulk density was determined after weighing soil samples taken intact from the field with a  $5 \times 5$  cm (diameter  $\times$  height) core. Total soil C and N in the samples were analysed on an Elementar Vario ES CN Analyser (Hanau, Germany). Soil available N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) was extracted at 1:10 with 2 M KCl and measured colorimetrically [19] by a Skalar autoanalyser. Soil was digested with acid mixture (6:1  $\text{HNO}_3\text{:HClO}_4$ ) and total P was measured colorimetrically according to Murphy and Riley [20]. Microbial biomass P was calculated from the difference between P concentration of hexanol-fumigated (microbial and soluble P) and non-fumigated (soluble P) samples [21]. Flushed P was extracted in de-ionised water by an anion exchange resin membrane. Two sub-samples of 10 g soil were prepared. One sub-sample was fumigated with chloroform in a desiccator for 24 h. After addition of 40 ml 0.5 M  $\text{K}_2\text{SO}_4$ , fumigated and non-fumigated samples were placed on an end-over-end shaker for 1 h and filtered through Whatman no. 42. Extracts were diluted with MilliQ water when required and analysed for dissolved organic C and N in a Formacs Series Combustion TOC/TN Analyser (Skalar, The Netherlands). The concentration of microbial C and N was calculated from the difference between the concentrations in extracts of fumigated (flushed and microbial C and N) and non-fumigated (flushed C and N) soil samples [22].

### 2.2. Soil salinisation

Three levels of salinity, denoted S1, S2 and S3, were induced in soil by addition of 0, 117 and 170 mg NaCl and 0, 588 and 1255 mg

$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$   $\text{kg}^{-1}$  soil in S1, S2 and S3, respectively, to maintain a low sodium adsorption ratio ( $\text{SAR} \leq 1$ ). The selection of the salinity levels was based on studies [1] indicating the positive effect of N addition on wheat growth. The salt solutions were thoroughly mixed with the soil using a cement mixer. The soils were incubated in the dark at  $25^\circ \text{C}$  at 50% of soil water holding capacity (WHC) to allow the microbial activity to equilibrate after rewetting of air-dried soil. After incubation, soil  $\text{pH}_{1:5}$  (soil: water suspension) was 6.29, 6.11 and 6.05 and average values ( $n = 4$ ) of  $\text{EC}_{1:5}$  (soil: water extract) were 0.21, 0.51 and 0.85  $\text{dS m}^{-1}$ , equivalent to  $\text{EC}_e$  (electrical conductivity in the extract of the saturated soil paste) 2.8, 7.1 and 11.9  $\text{dS m}^{-1}$ , in S1, S2 and S3, respectively.  $\text{EC}_e$  values were calculated using a conversion factor based on the relationship between the texture and the moisture of sandy loam soils [23].

### 2.3. Nitrogen and residue addition

After incubation, solutions of  $\text{KNO}_3$  or  $(\text{NH}_4)_2\text{SO}_4$  were added at 50 (N50)  $\mu\text{g N g}^{-1}$  soil to the saline sandy loam. A treatment without N (NO) was also included.

Residues of lupin (*Lupinus albus*, L.), ground to  $\leq 1.5$  mm, were added to soil at 2% (w/w). A control soil without residues was also included. Total C, N and P in residue were measured following the methods applied for soil analysis. Concentrations of lipids, lignin and cellulose in the residues were determined using  $^{13}\text{C}$  nuclear magnetic resonance (NMR). Lupin residue contained 40.6% total C, 2.64% total N, 0.11% total P, with a C/N ratio of 15.4 and a C/P ratio of 369. Lupin residue contained 10.8, 4.0 and 75.3% total lipids, lignin and cellulose, respectively. Residue amended and unamended (control) soils (20 g soil; dry weight equivalent) were filled into small containers with a mesh at the bottom for continuous measurement of soil respiration. Also, 250 g soil (dry weight equivalent) with and without plant residues were filled in pots for destructive sampling and determination of nutrients and microbial biomass. Throughout the experiment, the moisture of the soils in the pots and the small containers was maintained at 65% of WHC by adjusting the container weight with de-ionised water regularly.

### 2.4. Measurements

#### 2.4.1. Soil respiration

The small containers were placed individually in Mason jars containing a small container with 8 ml de-ionised water. The jars were closed with an air-tight lid with a septum and incubated in the dark in a constant temperature room ( $28 \pm 3^\circ \text{C}$ ). Released  $\text{CO}_2\text{-C}$  was measured using a gas analyser (Servomex 1450 series foodpack) by withdrawing air from the headspace with a needle through the lid's septum. After each measurement, the jars were opened to replace the  $\text{CO}_2$ -enriched air inside the jars with fresh air. The baseline of  $\text{CO}_2\text{-C}$  concentration was measured immediately after closing the jars. Soil respiration was measured over 45 days and cumulative respiration is expressed as  $\mu\text{g CO}_2\text{-C g}^{-1}$  soil.

#### 2.4.2. Microbial biomass and N and P availability

In the uncovered pots, soil sub-samples were taken on days 15, 30 and 45 for determination of microbial biomass C, N and P and available P and on days 20 and 40 for the measurement of available N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ). All measured parameters were expressed in  $\mu\text{g g}^{-1}$  soil.

### 2.5. Design and statistical analysis

The experiment was set up in a completely randomised design (3 salinity, 2 residue rate, 3 nitrogen) with four replicates. Two- and three-way analysis of variance and Tukey test were carried out

**Table 1**  
Characteristics of soils collected from Monarto in South Australia.

Parameters	Sandy loam
Sand (%)	75
Silt (%)	5
Clay (%)	20
$\text{pH}_{1:5}$ suspension	7.0
$\text{EC}_{1:5}$ ( $\text{dS m}^{-1}$ )	0.03
CEC [ $\text{cmol}(+) \text{kg}^{-1}$ soil]	13.0
ESP (exchangeable sodium percentage)	2.1
Bulk density ( $\text{g cm}^{-3}$ )	1.47
Water holding capacity (%)	19.8
Total C (%)	0.7
Microbial C ( $\mu\text{g g}^{-1}$ soil)	223.1
Total N (%)	0.03
Available $\text{NH}_4\text{-N}$ ( $\mu\text{g g}^{-1}$ soil)	14.4
Available $\text{NO}_3\text{-N}$ ( $\mu\text{g g}^{-1}$ soil)	8.0
Microbial N ( $\mu\text{g g}^{-1}$ soil)	26.5
Total P ( $\mu\text{g g}^{-1}$ soil)	148.0
Available P ( $\mu\text{g g}^{-1}$ soil)	2.3
Microbial P ( $\mu\text{g g}^{-1}$ soil)	3.4

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