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Symbiotic nitrogen fixation and soil N availability under legume crops in an arid environment

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Abstract
Purpose Legume crops often present an important option to maintain and improve soil nitrogen (N) quality and fertility in a dryland agroecosystem. However, the work on the integral assessment of the symbiotic N_2 fixation (N_{fix}) and their effects on soil N availability under field conditions is scare.
Materials and methods Five treatments consisted of legumes (capello woolly pod vetch and field pea), two non-legume crops (Saia oat and Indian mustard), and a nil-crop treatment as control (CK) in southeastern Australia to investigate the effects of legume crops on the amount of $N_{fix,}$ which was estimated using a ^{15}N natural abundance technique, and soil N pools, microbial biomass, microbial quotient, soil basal respiration, metabolic quotient (qCO_2), net N mineralization rates, and substrate-induced respiration (SIR) using the MicroResp method.
Results and discussion Crop ^{15}N natural abundances under legume crops were lower, and the amounts of N_{fix} in the aboveground vetch and pea biomass were 42.1 and 37.3 kg ha^{-1}, respectively, compared with the reference crops (oat and mustard). The crop treatments had higher soil pH, and lower moisture, NH_4^+–N and NO_3^-–N contents compared with the CK. The NO_3^-–N was predominant form of soil inorganic N across the treatments. Although no significant differences were found in microbial biomass carbon (C) and N across the treatments, legume crops had lower soil basal respiration and metabolic quotient, indicating that soil organic carbon was less easily accessible to microorganisms in comparison with the non-legume crops. In addition, no pronounced differences were found in soil available N pools (NH_4^+–N, NO_3^-–N, and soil soluble organic N) among the crop treatments. However, legume crops had lower soil net N mineralization rates and SIR, indicating lower soil potential N availability compared with the non-legume crops. These results showed that the amounts of N_{fix} by legume crops did not have immediate effects on soil N availability.
Conclusions Compared with non-legume crops, legume crops exerted less negative effects on the soil microbial properties in this dry environment. However, the amount of N_{fix} under legume crops did not immediately increase soil N availability over the growing season.

Keywords Legume · Microbial activity · Nitrogen fixation · Non-legume · Soil N availability

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1 Introduction

Nitrogen (N) is often the most important factor in determining crop productivity in agricultural ecosystems. Legume crops can fix N_2 from atmosphere (N_{fix}) and present an important option to improve N supply and to maintain soil fertility (Buerkert et al. 2000). In agricultural

ecosystems of temperate Australia, cover crop species such as pea and vetch are grown to control weeds during the growth period in winter and crop biomass will be applied on the ground to improve soil quality after cover crop harvest. Many studies have shown that legume crops can fix N under both field and laboratory conditions (Bilgo et al. 2007; Wichern et al. 2008). The amount of N_{fix} can be measured using enriched ^{15}N isotope dilution and natural abundance techniques compared with reference plants (Oberson et al. 2007). Studies have reported that crop ^{15}N natural abundances can provide more accurate estimates of the amount of N_{fix} (Oberson et al. 2007; Cheng 2009). However, the ability of N_2 fixation will be greatly affected in an arid environment, because crop growth can use water from the soil, subsequently limiting microbial activities for symbiotic N_2 fixation (Formowitz et al. 2009). Up to now little information is available about the effects of legume crops on the amount of N_{fix} in an arid environment, especially in temperate regions of southeastern Australia.

In addition, legume crops can greatly affect soil organic matter (SOM) and N cycling via crop litter decomposition and root exudation (Bever 2003). The SOM is a key component of soil fertility as a sink and source for nutrients (Xu et al. 2009). Soil labile organic pools, like microbial biomass and water soluble extracts, can act as the active or labile fractions of SOM and may indicate the potential microbial activity, which is sensitive to land uses and managements (Schloter et al. 2003). Studies have shown that these fractions are closely associated with crop productivity, because they provide available nutrients to plants (Frazão et al. 2010). Soil labile organic carbon (C) and N have been found to be strongly influenced by management practices and acted as indicators of soil quality (Chen and Xu 2008). Soil microbial biomass is critical in regulating soil ecosystem level processes and organic matter decomposition (Chen et al. 2004). Since soil microbial activity contributes to the regulation of soil C storage, metabolic quotient (qCO_2) provides a tool to relate both the size and activity of soil microbial communities (Odum 1969). Thus, it is widely employed as a parameter of the microbial activities in soils (Wardle and Ghani 1995).

Previous studies have shown that the growth of legume crops can increase soil N quality measured by ^{15}N labeling (Nguyen 2003; Wichern et al. 2008). Substrate-induced respiration (SIR) using the MicroResp method provides an option to study the soil N availability under legumes (Chapman et al. 2007). The MicroResp method, an advancement of Degens (1999) approach, reflects an immediate activity and provides physiological information on the soil microbial community substrate utilization pattern, which is similar to that obtained from Biolog methods (Campbell et al. 2003). Moreover, the estimates of respiration in response to added substrates correlate well with the actual size of microbial populations (e.g., Pennanen et al. 2004).

To our knowledge, little information is available about the work on the integral assessment of the N_{fix} and their effects on soil N availability under field conditions. The objectives of this study were to (1) quantify the amount of the N_{fix} in the aboveground legume biomass compared with non-legumes using ^{15}N natural abundances over a winter growing season, and (2) assess soil N availability and associated microbial activity under different crop treatments in southeastern Australia.

2 Materials and methods

2.1 Experimental site

The research was conducted at the Wagga Agricultural Institute in NSW, southeastern Australia (147°20′ E, 35°05′ S). The climate in this area is temperate with mean daily maximum temperatures ranging from 12.5°C in July (midwinter) to 31.2°C in January (mid-summer). Long-term records indicate mean annual rainfall of 550 mm, whereas 2009 was a dry year with 389 mm recorded (Fig. 1). The soil is a Chromic Luvisol and contains about 45% sand, 20% silt, and 35% clay. The study site has been cropped with winter wheat through May to December for years before 2005 and has been fallowed prior to the experiment. There were five treatments: Saia oat (*Avena strigosa*; designated as oat), capello woolly pod vetch (*Vicia villosa*; designated as vetch), field pea (*Pisum sativum*; designated as pea), Indian mustard (*Brassica juncea*; designated as mustard), and a nil-crop control (CK). Treatments were arranged in a randomized complete block design with three replications. Each plot was 40 m^2 (4× 10 m) with a rowing space of 22 cm. The crop species were broadcast-seeded at 80, 100, and 5 kg ha^{-1} for oat, pea and mustard, respectively on May 29th, 2009. Di-ammonium phosphate was applied at a rate of 80 kg ha^{-1} (including 20 kg N, 18 kg P, and 2–3 kg S ha^{-1}) for all plots at sowing.

2.2 Crop biomass assessment and soil sampling

The aboveground biomass of each crop was measured on October 9th, 2009 by cutting at the ground level from two random quadrats of 1 m^2 in each plot. The biomass was then combined and oven-dried at 70°C for 3 days. Soil samples were collected on the same day by taking five random cores (5-cm in diameter) to a depth of 10 cm in each plot. The five soil cores were immediately mixed thoroughly and kept in a cooler (ca. 4°C). After sieving (2 mm), the soil samples were stored at 4°C prior to analysis.

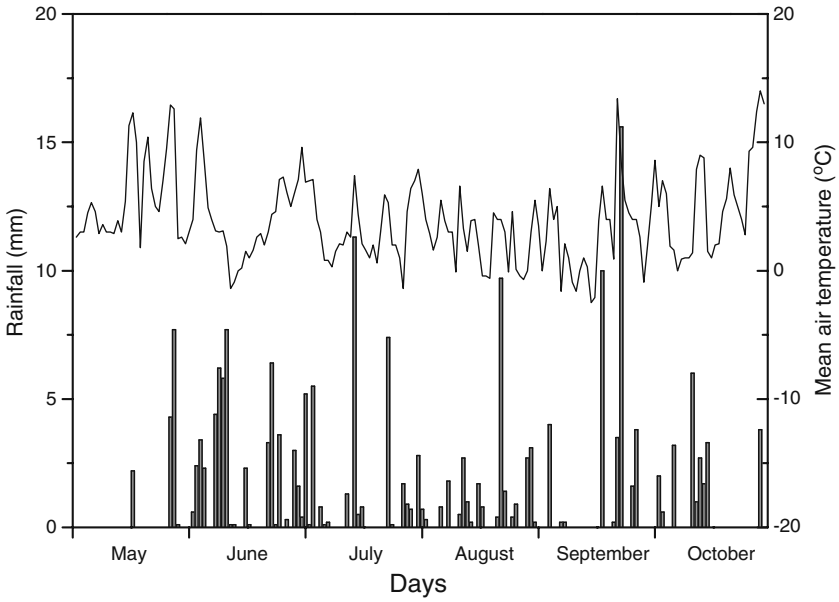

Fig. 1 Rainfall (*bars*) and mean air temperature (*line*) during the growing season from May 1st, 2009 to October 31th, 2009 at the experimental site in southeastern Australia

2.3 Crop ^{15}N and calculation of N_{fix} in aboveground legume biomass

Crop samples were finely ground and then ^{15}N natural abundance, total C and N contents were determined using an Isoprime isotope ratio mass spectrometer with a Euro-vector elemental analyzer (Isoprime-EuroEA 3000). Nitrogen isotope ratios are expressed in a relative δ notation:

$$\delta = [(R_{\mathrm{SAMPLE}} - R_{\mathrm{STD}})/R_{\mathrm{STD}}]^{*}1{,}000, \quad (1)$$

where R_{SAMPLE} is the $^{15}N/^{14}N$ ratio of the sample and R_{STD} is the $^{15}N/^{14}N$ ratio of atmospheric N_2 (Mariotti 1983).

The amounts of N_{fix} in the aboveground legume biomass were estimated using the following two-end-member separation equation:

$$N_{\mathrm{fix}} = N_{\mathrm{legume}}\left(\delta_{\mathrm{ref}} - \delta_{\mathrm{legume}}\right)/(\delta_{\mathrm{ref}} + \Delta), \quad (2)$$

where N_{fix} is the N fixed by the legume-Bradrhizobium system; N_{legume} is the amount of total N in the aboveground legume (pea and vetch) biomass; δ_{ref} is the $\delta^{15}N$ value of the aboveground reference crops (oat and mustard) biomass; δ_{legume} is the $\delta^{15}N$ value of the aboveground legume biomass; and Δ is the isotope fractionation factor associated with N_2-fixation for legume crops which is 0.3‰ (Unkovich et al. 1995).

2.4 Soil physical and chemical analyses

Soil moisture content was determined after being oven-dried at 105°C overnight. Soil pH was measured at a 1:2.5 soil/water ratio. Inorganic N and soluble organic N were measured by 2 M KCl extraction. In brief, a 5 g (dry weight equivalent) of soil samples was extracted with 30 ml of 2 M KCl in an end-to-end shaker for 1 h and filtered through a Whatman No. 42 paper. Concentration of inorganic N was measured using a Lachat Quickchem automated analyzer (Quick Chem method 10-107-064-D for NH_4^+ and 10107-04-1-H for NO_3^- and NO_2^-). Soil soluble organic C and total soluble N in soil extracts were determined using SHIMADZU TOC-$_{VCPH/CPN}$ analyzer (fitted with a TN unit) as described by Chen et al. (2004).

2.5 Analyses of soil microbial biomass and activity

Microbial biomass C (MBC) and N (MBN) were measured by the chloroform fumigation-extraction method using an EC factor of 2.64 (Vance et al. 1987) for MBC and an EN factor of 2.22 for MBN (Brookes et al. 1985). Briefly, two portions of 10 g field moist soil samples were weighed, and one portion of them was fumigated with chloroform for 24 h and extracted with 0.5 M K_2SO_4 in an end-to-end shaker for 1 h, and then filtered through a Whatman no. 42 paper. The other proportion of soil was directly extracted as above. The amounts of total soluble organic C and total soluble N in the fumigated and un-fumigated soil extracts were determined using SHIMADZU TOC-$_{VCPH/CPN}$ analyzer (fitted with a TN unit). Microbial quotient was the ratio of MBC to soil total C.

Soil basal respiration was calculated by a 33-day incubation as described by Chen et al. (2004). About 30 g (dry weight equivalent) of field moisture soil samples was incubated aerobically in a 1-L sealed jar at 22°C for 33 days in the dark. The CO_2 evolved from soils over the incubation period (1, 3, 6, 13, 20, 27, and 33 days) was trapped in

0.1 M NaOH and measured using 0.05 M HCl titration prior to the precipitation of carbonate with 1 ml of 1 M $BaCl_2$. The amounts of CO_2–C were calculated by the cumulative production of CO_2 from the soils during the incubation and qCO_2 was calculated as basal respiration over this period per mg of microbial biomass C (Wardle and Ghani 1995).

2.6 Soil net N mineralization rates and SIR

Soil net N mineralization rates were determined by a 7-day anaerobic incubation under laboratory conditions as described by Chen et al. (2002). Briefly, two portions of 5 g field moist soil samples were weighed, and one portion of them was added with 20 ml of distilled water and incubated at 40°C for 7 days, extracted with 20 ml of 4 M KCl in an end-to-end shaker for 1 h, and then filtered through a Whatman no. 42 paper. The other proportion of soil was directly extracted as above. Concentration of NH_4^+–N was measured using a Lachat Quickchem automated analyzer as described above.

Analysis of SIR for each treatment was performed using the MicroResp method as described by Campbell et al. (2003). In brief, sieved soil (<2 mm) was pre-incubated at 40% water holding capacity for 7 days prior to the metabolic assay. Soil was delivered to a deep-well microplate (0.20 g per well) containing a solution of predispensed C sources in relevant wells. The control was performed in wells with only water added (25 μl). SIR was measured by adding six different C sources, applied in 25 μl to achieve a final concentration of 30 mg g^{-1} soil water. The C sources included two carbohydrates (D-glucose and D-fructose), two amino acids (L-arginine and L-lysine), and two carboxylic acids (citric acid and malic acid), common exudates found in the plant roots (Chapman et al. 2007). The absorbance value was measured twice at 590 nm using ELISA plate reader, one immediately prior to sealing the soil microplate, and the other at 6-h incubation at 22°C. Each plate contained two soil samples with three replicates for each N source. Absorbance values were converted to CO_2 concentration, following the construction of a standard curve (Campbell et al. 2003).

2.7 Statistical analyses

One-way analysis of variance (ANOVA) was employed to determine the effect of crop species on crop biomass and ^{15}N natural abundances, soil N pools, microbial biomass, microbial quotient, qCO_2, net N mineralization rates and SIR. Correlations between soil moisture contents and soil basal respiration as well as qCO_2 were analyzed based on Pearson correlation coefficients ($P<0.05$). The differences were considered significant at $P<0.05$. All ANOVA, PCA and correlation analyses were performed using SPSS 12.0 software (SPSS Inc., USA).

3 Results

3.1 Crop ^{15}N natural abundance and N_{fix} in aboveground legume biomass

Crop ^{15}N natural abundance was the highest in the oat treatment, followed by the mustard, vetch and pea treatments (Fig. 2a). Crop ^{15}N natural abundances were significantly lower in the pea treatment than those in the

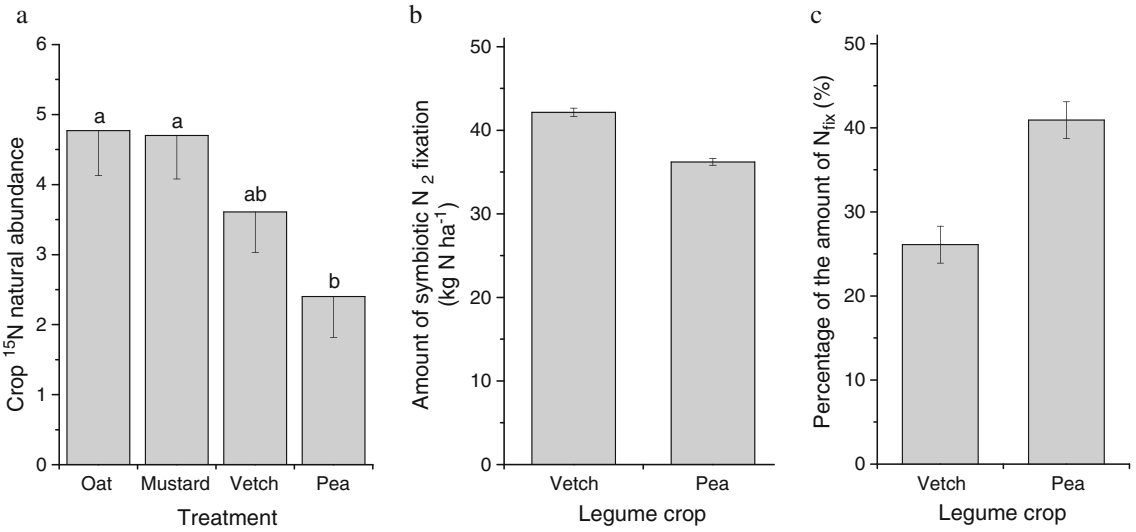

Fig. 2 Crop ^{15}N natural abundance (**a**), amount of symbiotic N_2 fixation (**b**) and percentage of the amount of N_{fix} in aboveground legume biomass N (**c**) under different crop treatments. *Different letters* showed significant differences at $P<0.05$

Table 1 Crop properties under different crop treatments

Treatment	Crop biomass (kg ha^{-1})	Total C (%)	Total N (%)
Oat	4,159±39 a, b	42.5±0.18	2.38±0.22 b
Mustard	3,514±25 b	43.1±0.58	2.48±0.18 a, b
Vetch	5,143±29 a	41.5±1.55	3.14±0.25 a
Pea	5,127±26 a	41.4±0.73	1.78±0.25 b

Data represent mean and standard error (n=3)

oat and mustard treatments. The oat and mustard treatments had similar crop ^{15}N natural abundances with values of 4.77‰ and 4.70‰, respectively (see Fig. 2a), thus the average value of those was used as δ_{ref}. In addition, the legume treatments had the highest aboveground biomass, which was significantly higher than that that in the mustard treatment (Table 1). The vetch treatment had significantly higher crop total N content than the mustard and pea treatments, but no significant differences were found in crop total C contents among the treatments (see Table 1). The amounts of N_{fix} in the aboveground vetch and pea biomass were 42.1 and 37.3 kg ha^{-1} (Fig. 2b), respectively, as compared with the reference crops. The amounts of N_{fix} in the vetch and pea treatments accounted for 26.1% and 40.9% of the aboveground biomass N, respectively (Fig. 2c).

3.2 Soil physical and chemical properties

The crop treatments had significantly higher soil pH, and lower moisture and NO_3^-–N contents compared with the CK (Table 2). With the exception of the oat treatment, the other crop treatments had significantly lower NH_4^+–N in comparison with the CK. Concentrations of inorganic N (NH_4^+–N plus NO_3^-–N) were significantly lower in the crop treatments compared with the CK. The inorganic N was predominated by NO_3^-–N across the treatments (see Table 2). There were no significant differences in soil total C and N contents among the treatments. Among the crop treatments, there were no significant differences in soil pH, moisture, total C and N contents. The oat treatment had the highest NH_4^+–N content, being significantly higher than the other crop treatments, while the mustard treatment had the highest NO_3^-–N content, being significantly higher than the oat and vetch treatments.

3.3 Soil soluble organic C and N and microbial biomass

No significant differences were found in soil soluble organic C across the treatments (see Table 2). The crop treatments had significantly higher soil soluble organic N compared with the CK, while no significant differences were found among the crop treatments (see Table 2). Similarly, no significant differences were found in microbial biomass C (Fig. 3a) and N (Fig. 3b) across the treatments. Microbial quotient, i.e., the ratio of MBC/total C, varied between 1.9 (oat) and 2.2 (pea). The pea treatment presented a significantly higher ratio than the oat treatment (Fig. 3c).

3.4 Soil basal respiration and qCO_2

The non-legume crops had significantly higher soil basal respiration than the other treatments (Fig. 3d). The oat treatment had the highest qCO_2, being significantly higher the other treatments (Fig. 3e). The qCO_2 was similar between the legumes and CK treatments, but was significantly higher in the mustard treatment than in the pea and CK treatments. Soil moisture contents were negatively correlated with soil basal respiration (r=−0.82; P<0.05; n=15) and qCO_2 (r=−0.69; P<0.05; n=15).

3.5 Soil net N mineralization rates and SIR

The legume treatments tended to have lower soil net N mineralization rates (Fig. 4). With the exception of the pea

Table 2 Soil properties with and without crop species

Treatment	pH	Moisture (%)	NH_4^+–N (mg kg^{-1})	NO_3^-–N (mg kg^{-1})	Soluble organic C (mg kg^{-1})	Soluble organic N (mg kg^{-1})	Total C (%)	Total N (%)
Oat	5.0±0.05 a	5.3±0.4 b	1.64±0.1 a	2.1±0.1 c	72±2.6	7.4±0.6 a	2.06±0.09	0.18±0.01
Mustard	5.0±0.02 a	5.9±0.1 b	0.05±0.01 c	6.9±0.4 b	68±3.9	7.7±0.4 a	2.03±0.07	0.18±0.01
Vetch	5.1±0.08 a	5.5±0.6 b	0.43±0.08 b	2.5±0.7 c	70±2.7	8.8±0.2 a	2.04±0.17	0.18±0.02
Pea	5.0±0.07 a	7.3±0.9 b	0.88±0.04 b	4.5±0.7 b, c	70±2.1	6.3±0.4 a	2.10±0.13	0.18±0.01
CK	4.6±0.05 b	11.1±0.4 a	1.5±0.08 a	35.4±2.2 a	77±2.6	1.2±0.1 b	1.96±0.12	0.17±0.01

Data represent mean and standard error (n=3)

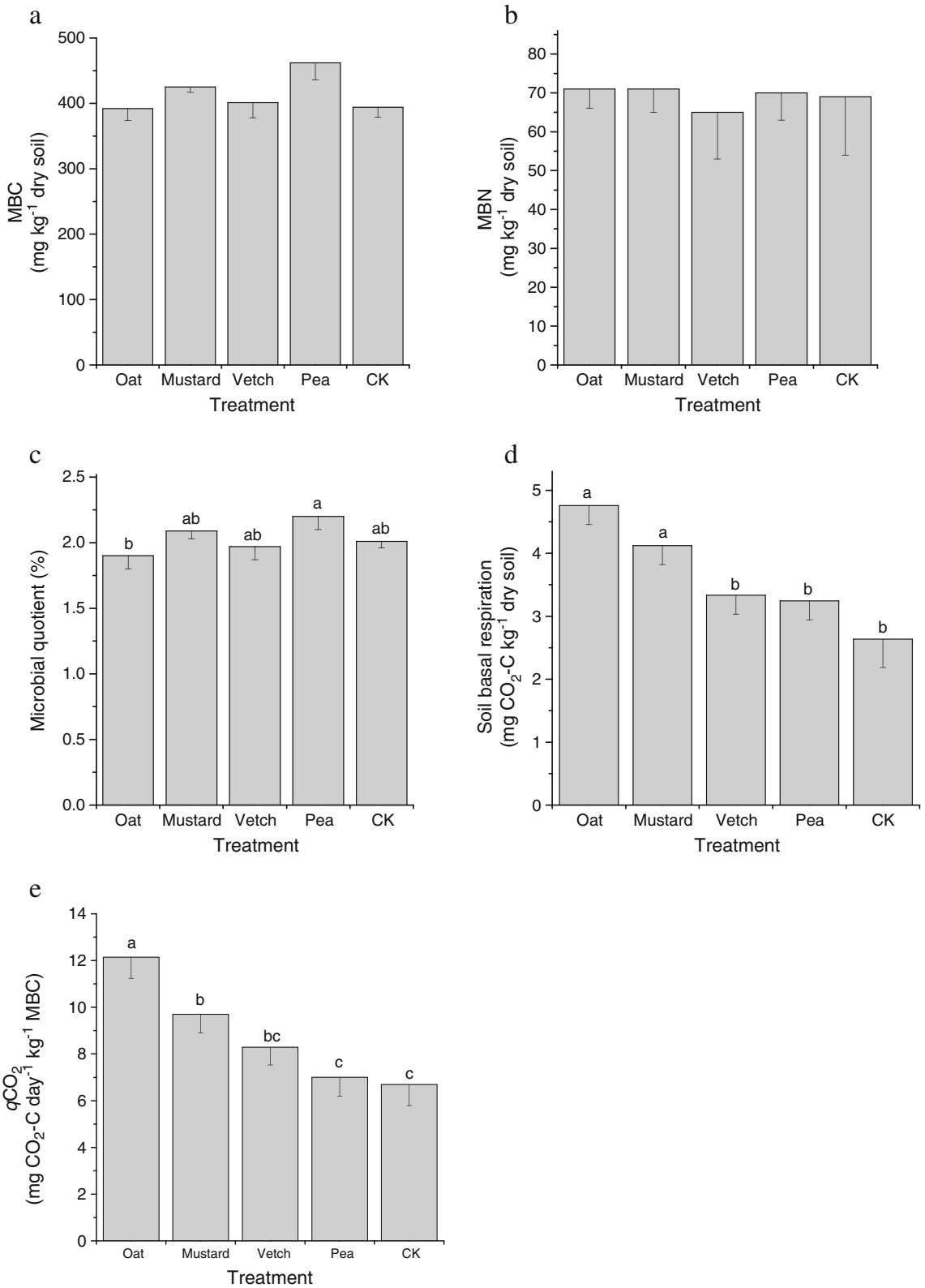

Fig. 3 Microbial biomass C (*MBC*) (**a**) and N (*MBN*) (**b**), microbial quotient (MBC/Total C) (**c**), soil basal respiration (**d**) and metabolic quotient (qCO_2) (**e**) under different crop treatments. Data represent mean and standard error (n=3). *Different letters* indicated significant differences at $P<0.05$

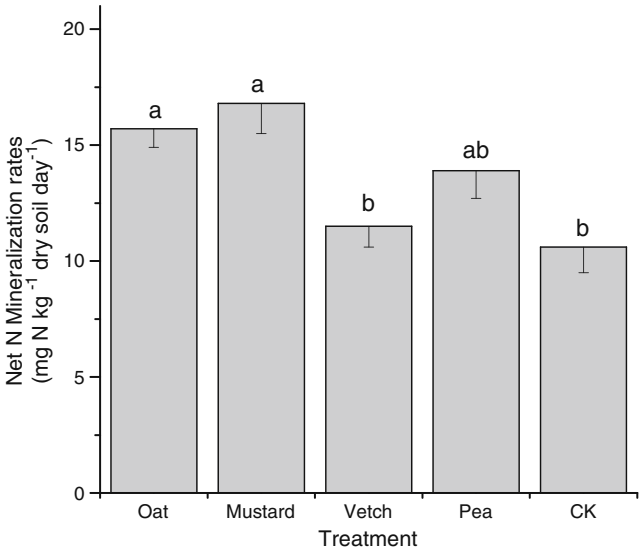

Fig. 4 Soil net N mineralization rates under different crop treatments. *Different letters* indicated significant differences at $P<0.05$

treatment, the non-legume treatments had significantly higher net N mineralization rates than the vetch and CK treatments. Net N mineralization rates did not differ between the legumes and CK treatments.

Significant differences in SIR were observed with the addition of different C sources, the highest values with citric acid (Fig. 5). Significant differences in SIR were found with the addition of two amino acids and two carboxylic acids across the treatments, but no pronounced differences were found with two carbohydrates. In general, the SIR was higher in the non-legume and CK treatments than in the legume treatments. Considering the sum of SIR for each treatment, we found that the total SIR decreased in order of the oat>CK>mustard>vetch>pea treatments.

4 Discussion

4.1 Legume N fixation and soil inorganic N availability

Results from this study had demonstrated that legume crops had the ability to fix N_2 from atmosphere in this arid environment (see Fig. 2b). Previous studies showed that the total amount of N_{fix} varied greatly with different legumes and different management practices such as fertilization. Studies reported that the range of N_{fix} was between 150 and 260 kg ha^{-1} for the whole soybean (Oberson et al. 2007), while it was reported to be between 54 and 165 kg ha^{-1} for the field pea shoot with a mean of 144 kg ha^{-1} (Unkovich et al. 1995). In this study, the amounts of N_{fix} in the aboveground legume biomass were much lower (about 40 kg ha^{-1}; see Fig. 2b), but the percentage of legume N derived from the atmosphere (see Fig. 2c) was similar to previous studies (Carranca et al. 1999; Oberson et al. 2007). The reason for the lower amounts of N_{fix} was largely attributed to lower aboveground legume biomass in the dry environment, which was about 5,100 kg ha^{-1} as compared with that of about 18,000 kg ha^{-1} under favorable conditions (Oberson et al. 2007). However, the amounts of N_{fix} in the legume treatments were similar to what was reported (4–38 kg ha^{-1}) under drought stress in a Mediterranean environment (Carranca et al. 1999).

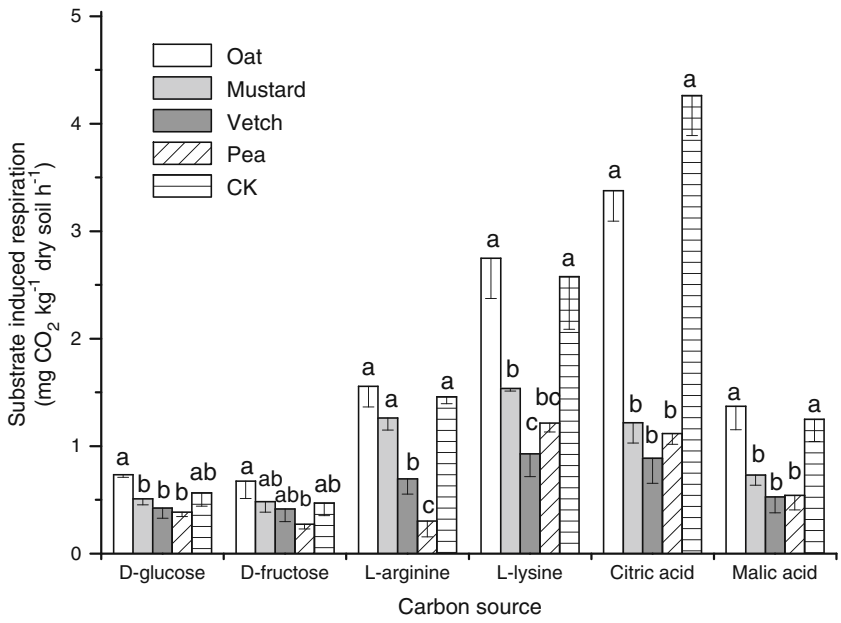

Fig. 5 Substrate-induced respiration with addition of different carbon sources under different crop treatments. *Different letters* indicated significant differences at $P<0.05$

The form of soil inorganic N was dominated by NO_3^-–N across the treatments (see Table 2), indicating the strong nitrification and weak denitrification in the soils under the arid environment. The presence of the large amount of NO_3^-–N in the CK treatment presented a high potential of loss of N through leaching (Frazão et al. 2010), which was evidenced by our subsequent observations over 1 year (data not shown). The significantly higher amount of NO_3^-–N in the CK compared with the crop treatments could be attributed to the crop N uptake during the growing season. There were no pronounced differences in soil inorganic N availability between the legume and non-legume treatments (see Table 2). The reason for this could be the fact that majority of N fixed by legumes still remained in the crop biomass.

4.2 Soil labile organic C and N pools

Soil soluble organic C is an important pool with respect to soil organic matter turnover in agricultural ecosystems, since it functions as readily decomposable substrates for soil microorganisms and as a short-term nutrient reservoir for crop growth (Chen et al. 2004; Wichern et al. 2007). Some studies used microbial biomass as an indicator of soil organic carbon (SOC) (Degens and Sparling 1996), while others considered the KCl extractable organic C (Chen and Xu 2008).

No significant differences in microbial biomass were found among the treatments (see Fig. 2). The possible explanation was that (1) microbial growth was limited by the low soil water availability, which negatively controlled microbial activity, and (2) microbial biomass was decoupled with microbial activity (Geisseler and Horwarth 2009). Thus, attention should be paid when using microbial biomass as an indicator of SOC under dry environments. The microbial quotient was an index of the accumulation potential of microbial biomass C relative to SOC (Sparling 1992), which could be used to assess changes caused by agricultural practices (Wardle 1992). The pea treatment had higher microbial quotient, indicating that higher efficiency in conversion of organic C into microbial C.

4.3 Soil basal respiration and qCO_2

The higher soil basal respiration in the non-legume treatments suggested SOC was more easily accessible to microorganisms than in the legume treatments (Wardle and Ghani 1995). Soil C tied-up in microbial biomass in the legume treatments was increased and associated with reduced losses through microbial respiration.

The qCO_2 is originally based on Odum's theory of ecosystem succession (Odum 1969). An increase in qCO_2 has been interpreted as a microbial response to adverse environmental stress or disturbance (Wardle and Ghani 1995). A higher qCO_2 mean that less substrate C available will be incorporated into microbial biomass and higher C per unit MBC will be lost through respiration. The higher qCO_2 under crop species means that the growth of crops causes a negative effect on the soil microbial communities in this arid environment. Compared with the legumes, non-legumes had more severe disturbance on the soil microbial properties (see Fig. 3e). This was clearly supported by the negative relationships between soil moisture contents and qCO_2 ($r=-0.82$; $P<0.05$; $n=15$).

4.4 Soil net N mineralization rates and SIR

Mineralization of organic N to NH_4^+–N and NO_3^-–N takes place through microbial transformation processes and, subsequently provides available N to crops. The measurement of net N mineralization under the water-logging condition has been used as an index of soil potential N availability (Chen et al. 2002). Lower soil net mineralization rates in the legume treatments indicated lower soil potential N availability (see Fig. 4). The results were supplemented by the SIR with lower values in the legume treatments (see Fig. 5). The immediate respiration response measured by MicroResp was not supposed to change the community structure during the short incubation period (Pennanen et al. 2004). The SIR was directly influenced by the soil N pools and Lagomarsino et al. (2007) reported an increase in SIR values with fertilization. Although previous studies reported that the growth of legume crops could increase soil N quality (Bertin et al. 2003; Paterson 2003), similar amounts of N were released into the soils under legumes in comparison with non-legumes (16% vs 14%) (Nguyen 2003; Wichern et al. 2008).

5 Conclusions

Legume crops have the ability of symbiotic N_2 fixation in this arid environment of temperate Australia. The NO_3^-–N was predominant form of soil inorganic N across the treatments, which may present a risk of N loss through leaching. The growth of crop species utilized water from the soils and caused a negative effect on the soil microbial properties. Legume crops had lower soil basal respiration and metabolic quotient, indicating that SOC was less easily accessible to microorganisms and less negative effects on the soil microbial properties in comparison with the non-legume crops. No pronounced differences were found in soil available N pools (NH_4^+–N, NO_3^-–N, and soil soluble organic N) among the crop treatments. However, legume crops had lower net N mineralization rates and SIR, which showed that the N_2 fixation by legume crops did not have immediate effects on soil N availability.

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