# Winter cover crops increase soil carbon and nitrogen cycling processes and microbial functional diversity

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## Abstract

Winter cover crops are not only one of effective agricultural management practices to control weeds but also can improve soil fertility, resulting in increasing crop productions. Up to now, however, little is known about information on how much of soil soluble organic carbon (C) incorporates into the soils applied with winter cover crops, which is a prerequisite to design strategies that improve C sequestration in agricultural ecosystems. The aims of this study were to: (1) assess the effects of winter cover crops on soluble organic carbon (SOC) pools using different extraction methods (KCl extractable organic C; microbial biomass) and microbial community functional diversity, and (2) quantify how much of the potentially mineralizable organic C pools ($C_0$) incorporates into the soils and associated half-life of SOC remaining under seven cover crops and nil-crop control (CK) in temperate agricultural soils of southern Australia. Cover crop treatments are cereal rye, wheat, saia oats, vetch, field peas, mustard and the mixture of cereal rye and vetch. Results showed that the CK treatment had higher soil moisture content and lower soluble organic nitrogen (SON) compared to the cover crop treatments. Among the cover crop treatments, there was significantly higher SON in the wheat, oats and vetch treatments than in the other treatments. The oats treatment had the highest amount of cumulative $CO_2$-C than any other treatments over one-month incubation experiment. An exponential regression approach for C mineralization was used to estimate $C_o$ and soil samples under the cover crops can be divided into four groups depending on $C_o$. The principal component analysis of the MicroResp$^{TM}$ profiles showed that the CK treatment was significantly different from the cover crop treatments. The cover crop treatments with wheat, vetch and peas as well as mustard form a cluster which was significantly different from the other clusters. In addition, the vetch, field peas and mustard treatments showed higher Shannon diversity H and Evenness (E) and Simpson diversity H compared to the other cover crop treatments with the lowest Shannon H and E at CK. In conclusion, overall, the vetch and field peas as well as wheat winter cover crop may be better management practices for agricultural ecosystems in southern Australia.

## Introduction

Many of studies have shown that cover crops are not only an effective agricultural management practice to control weed but also provide many services to agro-ecosystems, such as decreasing erosion, improving soil nutrient retention and building soil organic matter (SOM) (Carrera *et al.* 2007; Sainju *et al.* 2008). Most of these studies, however, have dealt with either single or a few cover crops and there are very few studies on an overall evaluation of the role of cover crops in the C sequestration by combining soil labile C and N pools and microbial functional diversity and cover crop biomass. Soil organic matter is an important ecosystem property and regarded as widely acknowledged indicator for soil quality (Chen *et al.* 2004). However, it can take many years to detect differences in SOM under different types of management. Labile fractions of SOM like SOC and SON are very important because they control ecosystem productivity in the short term and are more sensitive to management practices (Huang *et al.* 2008). A number of techniques, including KCl extraction and microbial biomass measurement by fumigation, have developed to fractionate labile C pools from SOM. Labile C pools comprise the easiest available sources of energy for microorganisms and therefore, it is useful to study when analyzing the soil microbial community functional diversity using MicroResp$^{TM}$ which has been shown to be more discriminatory than Biolog (Campbell *et al.* 2003). As SOC has been regarded as an indictor for soil quality and its decomposition is a function of different factors, an understanding of C mineralization in soils applied with cover crops is a prerequisite for predicting contributions of these SOC pools to global $CO_2$ balance and helping to develop strategies to sequester more SOC into these soils and to maintain soil functions. The aims of this study were to (1) assess the effects of winter cover crops on SOC pools using different extraction methods and microbial community functional diversity, and (2) quantify how much of the potentially mineralizable organic carbon pools ($C_0$) incorporate in soils and associated half-life of SOC remaining under seven cover crops compared to the nil-crop control (CK).

**Methods and materials**

The research was conducted in a full factorial design with seven cover crops, i.e., cereal rye, wheat, saia oats, vetch, field peas, mustard and mixture of cereal rye and vetch (designated as mixture) and the nil-crop control (CK) with three replicates in Wagga Wagga Institute in southern Australia. The area of each plot is 40 $m^2$ with a rowing space of 22 cm between plots. Seeds of cover crops were broadcast on 29$^{th}$ May 2009, with the sowing rate of 80 kg/ha for rye, 80 kg/ha for wheat, 80 kg/ha for oats, 50 kg/ha for vetch, 100 kg/ha for peas, 5 kg/ha for mustard and 45 (cereal rye) and 40 (vetch) kg/ha for the mixture. Fertilizer applied on all plots on the same day of broadcasting was diammonia phosphate at a rate of 80 kg/ha, including 20 kg N, 18 kg P and 2-3 kg S/ha. Soil samples were collected on 9$^{th}$ Oct 2009 after the cover crops were harvested. Five cores were taken to a depth of 10 cm in each plot. The concentration of inorganic N was measured a Lachat Quickchem automated ion analyzer. SOC and SON were extracted using KCl extraction method, described by Huang *et al.* (2008). Microbial biomass was measured by chloroform fumigation-extraction method (Chen *et al.* 2004). Soil microbial community functional diversity was measured using the MicroResp$^{TM}$ system with the application of 16 C sources (Campbell *et al.* 2003). Plant aboveground biomass was measured by clear cutting at the ground level at a 1-$m^2$ quadrat placed in each plot. All ANOVA, regression and t-test analyses were performed using SPSS 11.0 software (SPSS Inc., USA). The microbial community diversities based on MicroResp$^{TM}$ were submitted to principal component analysis (PCA). To estimate the potentially mineralizable $C_0$ and first-order rate constant (k), the non-linear regression approach for N mineralization of Smith *et al.* (1980) was used: $C_m=C_0*(1-\exp^{-kt})$, where $C_m$ is the organic C mineralized (mg/kg) at a specific time (t).

**Results**

Table 1 shows some basic soil properties in soils under the cover crops. The CK treatment had significantly higher soil moisture content and lower pH and SON compared to the cover crop treatments. There were not significant differences in microbial biomass C (MBC) and N (MBN) among the treatments. Among the cover crops, SON was significantly higher in wheat, oats and vetch treatments than in others. Cover crop aboveground biomass was significantly lower in the field peas than in the other treatments. The cover crop with oats had the highest amount of cumulative $CO_2$-C evolved with the lowest amount in the CK treatment and the intermediate in the other treatments (Figure 1). By comparison of $C_0$ in the treatments, statistical analyses showed that the soils under the cover crops had significantly higher ability to release SOC as $CO_2$ compared to the CK. Four distinct groups of soils were distinguished for $C_0$ values: Group 1 included the cover crops with oats and field peas; Group 2 included the cover crops with wheat, vetch and mustard; Group 3 included the cover crops with cereal rye and mixture; Group 4 included the CK. The decomposition constant (k) and the time required to mineralize one-half of the potentially mineralizable C ($t_{1/2}$) are shown in Table 3. The average basal respiration measured using MicroResp$^{TM}$ ranged from 0.02 to 0.30 ug $CO_2$-C/g/h (Figure 2). All substrates induced respiration (SIR) above the basal respiration (water only). SIR for L-arginine, citric acid, malic acid, oxalic acid, D-fructose and D-glucose was significantly higher than the other C sources. The CK showed higher utilization capacities for L-arginine, citric acid and L-lysine. Based on MicroResp$^{TM}$, Shannon diversity index H and Evenness (E) we found H and E were significantly higher in the cover crops than in the CK (Table 4). Among the cover crops, the cover crops with vetch, field peas and mustard showed significantly higher Shannon H and E compared to the other treatments. In addition, Simpson diversity index H showed the similar trend with the highest H in the vetch and field peas treatments. The principal component analysis of the MicroResp$^{TM}$ profiles showed that CK treatment is significantly different from the cover crop treatments. The cover crops with wheat, vetch and peas as well as mustard form a cluster which was significantly different from the other clusters (Figure 3).

**Conclusion**

With overall evaluation of SOC and SON pools, $C_0$, cover crop production as well as microbial functional diversity index and evenness, the vetch and field peas as well as wheat winter cover crop may be better management practices for agricultural ecosystems in southern Australia.

**Table 1. Selected soil properties under different winter cover crops in southern Australia.**

| Treatments | pH | Soil moisture | SOC (mg/kg) | SON (mg/kg) | MBC (mg/kg) | MBN (mg/kg) |
|---|---|---|---|---|---|---|
| Cereal rye | 5.0±0.05a | 5.7±0.6b | 688±31ab | 9.7±0.2b | 426.5±22.4 | 78.9±4.7 |
| wheat | 5.1±0.06a | 6.4±0.7b | 730±8a | 14.2±0.6a | 430.9±31.0 | 84.0±11.1 |
| Saia oats | 5.0±0.05a | 5.3±0.4b | 710±38a | 12.2±0.8ab | 392.5±18.1 | 71.3±5.0 |
| Vetch | 5.1±0.08a | 5.5±0.6b | 710±36a | 14.6±0.4a | 401.8±73.0 | 65.3±12.1 |
| Field peas | 5.0±0.07a | 7.3±0.9b | 718±28a | 10.5±0.3b | 462.7±26.4 | 69.6±7.5 |
| Mustard | 5.0±0.02a | 5.9±0.1b | 688±24ab | 11.9±0.4ab | 425.1±8.0 | 70.9±6.2 |
| Mixture | 5.1±0.02a | 5.5±0.5b | 674±29ab | 10.9±0.8b | 455.3±22.7 | 74.0±10.7 |
| CK | 4.6±0.05b | 11.1±0.4a | 586±46b | 1.4±0.3c | 394.0±15.7 | 69.4±8.8 |

Data are mean ± S.E. (n=3). Means within a column followed by the same letter are not different at the 5% level of significance by t-test.

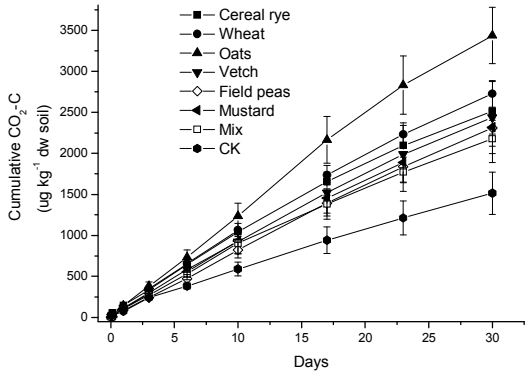


**Figure 1. Cumulative $CO_2$-C evolved from soils under cover crops in southern Australia.**

**Table 2. The properties and aboveground biomass of different cover crops in southern Australia.**

| Treatments | C (%) | N (%) | Biomass (kg/ha) | C:N ratio |
|---|---|---|---|---|
| Cereal rye | 40.9±1.8 | 1.67±0.22c | 3857±79ab | 25.36±1.73b |
| wheat | 41.1±0.4 | 1.35±0.12c | 4858±72a | 30.84±1.79a |
| Saia oats | 42.5±0.1 | 2.38±0.22b | 3930±34ab | 18.21±0.84c |
| Vetch | 41.5±3.5 | 3.14±0.23a | 4871±27a | 13.20±0.18d |
| Field peas | 41.4±0.7 | 1.77±0.25c | 4858±26a | 24.18±1.16b |
| Mustard | 43.1±0.5 | 2.47±0.18b | 3323±25b | 17.63±1.51b |
| Mixture | 42.7±0.1 | 1.78±0.23c | 4294±45ab | 24.88±1.37b |

Data are mean ± S.E. (n=3). Means within a column followed by the same letter are not different at the 5% level of significance by t-test.

**Table 3. Comparison of calculated potentially mineralizable organic C pools ($C_0$) and first order rate constants (k) and half life of C remaining in soils under cover crops in southern Australia.**

| | $C_o$ | k | $R^2$ | Half-life (day) |
|---|---|---|---|---|
| Cereal rye | 4.93c | 0.02395 | 0.99 | 29 |
| Wheat | 6.61b | 0.0178 | 0.99 | 39 |
| Oats | 12.59a | 0.01081 | 0.99 | 64 |
| Vetch | 6.66b | 0.01526 | 0.99 | 45 |
| Field Peas | 12.62a | 0.00677 | 0.99 | 102 |
| Mustard | 5.59b | 0.01786 | 0.99 | 39 |
| Mix | 4.74c | 0.02042 | 0.99 | 34 |
| CK | 3.45d | 0.01902 | 0.99 | 36 |

**Table 4. Shannon diversity index H and Evenness as well as Simpson diversity index H based on the calculation of microbial community functional diversity using MicroResp™ under different cover crops in southern Australia.**

| Treatments | Shannon H | Evenness | Simpson H |
|---|---|---|---|
| Cereal rye | 2.13±0.02c | 0.53±0.01cd | 0.72±0.02d |
| Wheat | 2.28±0.04b | 0.61±0.03b | 0.84±0.01b |
| Oats | 2.21±0.04b | 0.57±0.03bc | 0.83±0.01b |
| Vetch | 2.61±0.05a | 0.85±0.04a | 0.87±0.01a |
| Field Peas | 2.48±0.05a | 0.74±0.04a | 0.83±0.01b |
| Mustard | 2.52±0.02a | 0.78±0.02a | 0.87±0.01a |
| Mix | 2.17±0.04b | 0.55±0.02d | 0.82±0.01bc |
| CK | 1.93±0.02d | 0.43±0.01e | 0.80±0.01c |

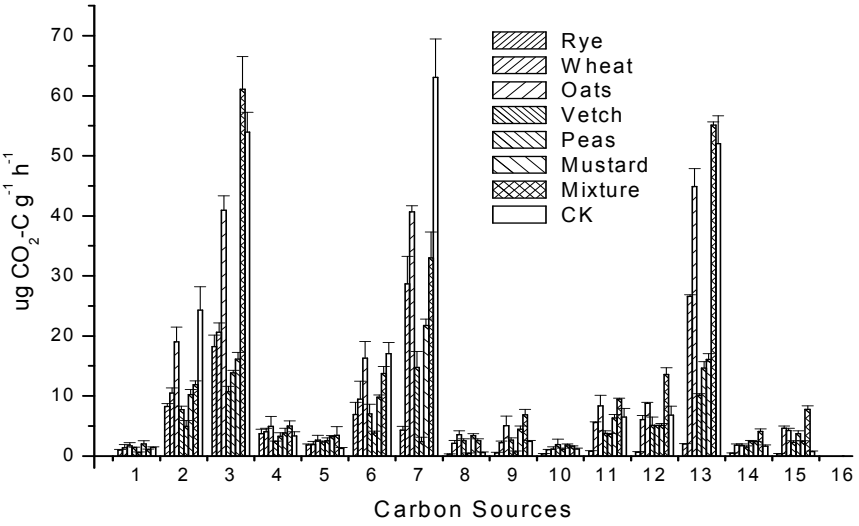


**Figure 2. Respirometric evolution of carbon dioxide after 6 h with or without the addition of 15 different C sources for soils under different cover crops in southern Australia. The numbers from 1 to 16 correspond to represent L-alanine, L-arginine, citric acid, D-galactose, amino butyric acid, malic acid, oxalic acid, 3,4-OH benzoic acid, L-arabinose, L-cysteine, D-fructose, D-glucose, L-lysine, N-acetyl glucosamine, D-trehalose, water, respectively.**

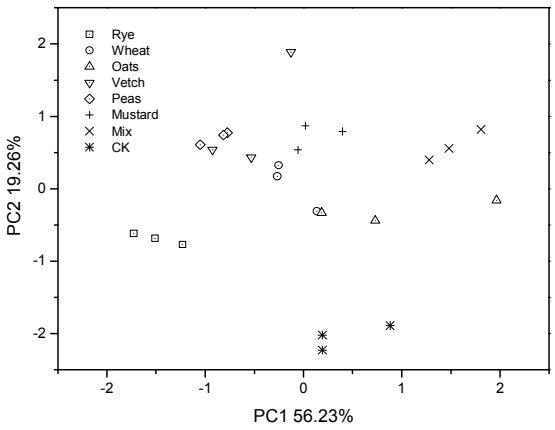


**Figure 3. Ordination plot of the first two canonical axes produced by principal component analysis (PCA) based on MicroResp™ C source utilization profiles for soils under different cover crops.**

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