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Differential Effects of Legume Species on the Recovery of Soil Microbial Communities, and Carbon and Nitrogen Contents, in Abandoned Fields of the Loess Plateau, China

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Abstract Plant–soil interactions are known to influence a wide range of ecosystem-level functions. Moreover, the recovery of these functions is of importance for the successful restoration of soils that have been degraded through intensive and/or inappropriate land use. Here, we assessed the effect of planting treatments commonly used to accelerate rates of grassland restoration, namely introduction of different legume species *Medicago sativa*, *Astragalus adsurgens*, *Melilotus suaveolens*, on the recovery of soil microbial communities and carbon and nitrogen contents in abandoned fields of the Loess Plateau, China. The results showed effects were species-specific, and either positive, neutral or negative depending on the measure and timescale. All legumes increased basal respiration and metabolic quotient and had a positive effect on activity and functional diversity of the soil microbial community, measured using Biolog EcoPlate. However, soil under *Astragalus adsurgens* had the highest activity and functional diversity relative to the other treatments. Soil carbon and nitrogen content and microbial biomass were effectively restored in 3–5 years by introducing *Medicago sativa* and *Astragalus adsurgens* into early abandoned fields. Soil carbon and nitrogen content were retarded in 3–5 years and microbial biomass was retarded in the fifth year by introducing *Melilotus suaveolens*. Overall, the restoration practices of planting legumes can significantly affect soil carbon and nitrogen contents, and the biomass, activity, and functional diversity of soil microbial community. Therefore, we propose certain legume species could be used to accelerate ecological restoration of degraded soils, hence assist in the protection and preservation of the environment.

Keywords Soil microbial biomass · Soil carbon · Functional diversity · Abandoned field · Legume species · Ecological restoration

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Introduction

It is well known that plant species differentially affect the size, composition and activity of soil biological communities, and that such differential effects influence ecosystem functions such as soil carbon (C) and nitrogen (N) storage in soil and the retention of nutrients and water in soil (Hooper and Vitousek 1998; Bardgett and others 2008; De Deyn and others 2009; De Vries and others 2012; Zeng and others 2009). Moreover, effects of plants on soil microbes can feedback to the plant community through the stimulation of nutrient mineralization processes or by promoting mycorrhizal fungi which enhance plant nutrient uptake, resulting in qualitative differences in plant community composition (Bardgett 2005; Kardol and others 2006; van der Heijden and others 2008). For instance, changes in plant species and functional group richness have been shown to influence the storage and loss of both C and N in model grassland communities, although these responses are related to the presence and biomass of certain plant species,

notably N-fixers and forbs (De Deyn and others 2009). As a result, plant–soil feedbacks are likely to play a key role in restoration processes in abandoned land, and as recently argued by Kardol and Wardle (2010), the integration of principles developed from research on aboveground–belowground linkages can greatly assist restoration ecology.

Cultivation of steep slopes using traditional technology including deep ploughing, harrowing, and rain-fed farming, has been ongoing for several centuries on Loess Plateau, China (Catt 2001; Liu and Ding 2004). Due to serious soil and water loss, the Chinese Government in 1999 proposed the Conversion of Cultivated Land to Grassland and Forest program which aims to reduce soil erosion and improve soil quality through the establishment of perennial grass cover on degraded arable land (Gan and Sang 2002). Through this program, 3.61 million hectares of sloping cultivated land are going to be abandoned gradually across the Loess Plateau. The current restoration program advocates the introduction of some legume species in the early abandoned fields to accelerate the rate of recovery of vegetation and soil properties (Xue and others 2002; Li and others 2007, 2008). However, our understanding of how different legume species affect soil properties, including the biomass and functioning of the soil microbial community, is limited.

Different legume species are often selected to conserve soil and water in arid and semi-arid regions of China for their strong capability to grow in poor conditions-drought, cold and poor soil. These include *Medicago sativa* Linn. (Fu 2001), a perennial herb, is cultivated almost worldwide. It can grow in a loess hill-gully region for 10 years, being most productive after 4 or 5 years since introduction, and then gradually declines because of the excessive depletion of soil water (Cheng and others 2005). *Astragalus adsurgens* Pall. (Fu 2001) is a perennial species, endemic to China. It grows for only 6–7 years in the loess hill-gully region, and its maximum biomass occurs after 3 or 4 years, and then its growth slows and biomass decreases due to reduced available soil moisture (Cheng and others 2004). Finally, *Melilotus suaveolens* Ledeb. (Ohashi and Tateishi 1984), an annual or biennial herb, is widely distributed in northern part of China and other countries. Although it is known that these species differentially affect soil nitrogenase activity, in the order of *Medicago sativa* > *Melilotus suaveolens* > *Astragalus adsurgens* (Wang and Hu 2001), little is known about how these species influence soil nutrient levels or microbial communities which, as mentioned above, play a key role in the restoration of degraded soils.

In the region of our study, the ultimate goal of restoration is to get abandoned land back to the native vegetation type, *Stipa breviflora* grasslands, as quickly as possible. Therefore, *Stipa breviflora* is the target species in the restored communities. The motivation for using legumes, however, is based on their ability to rapidly establish and facilitate the restoration of native vegetation (Zou and others 1998; Xue and others 2002; Li and others 2007, 2008), to aid soil stabilization, and to increase soil N availability (Rochon and others 2004; Hopkins and Wilkins 2006) and potentially soil C sequestration (Soussana and others 2004; Fornara and Tilman 2008; De Deyn and others 2009; De Deyn and others 2011). Also, Li and others (2008) reported weeds can be inhibited by the introduction of legume species, and *Melilotus suaveolens* can facilitate the growth and establishment of native grassland species, thereby accelerating the course of old-field succession. Despite these potential benefits, there have been few specific evaluations of the effects of introducing different legumes species on the recovery of soil properties and functions that underpin successful restoration programs.

Soil microbial communities are crucial to the functioning of soils. This is because soil microbes are responsible for establishing biogeochemical cycles. Soil microbial properties such as microbial biomass may be used as early and sensitive indicators of soil quality (Bending and others 2004). Soil basal respiration, a measure of soil organic matter decomposition, has been reported to be influenced by soil temperature, moisture (Liu and others 2005), C input (Landi and others 2006) and microbial biomass. The metabolic quotient (qCO_2) has been applied for the quantification of environmental effects on the microbial community in soils (Anderson and Domsch 1990; Brookes 1995; Wardle and Ghani 1995; Bååth 1998; Chodak and others 2009). In ecological terms, a high qCO_2 reflects a high maintenance of carbon demand, and if the soil system cannot replenish the carbon which is lost through respiration, microbial biomass must decline (Anderson and Domsch 2010). The ratio of MBC:TOC has been used as sensitive index of soil quality for management practices (Saviozzi and others 2001) and for accumulation of organic matter in soil (Sparling 1992). Changes in the MBC:MBN ratio have often been attributed to shifts in the structure of the soil microbial community (Jenkinson 1976; Paul and Clark 1996; Kara and Bolat 2008). Increasing contents of organic carbon and microbial biomass may result in increased functional diversity of soil microbial communities (Yan and others 2000) and thus increased functionality and stability of soil ecosystems (Degens and others 2001).

The purpose of this study was to investigate the influence of different legume species on soil microbial communities and C and N contents in abandoned fields on the central Loess Plateau, China. Specifically, we assessed the effect of different treatments that are typically used to accelerate rates of grassland restoration, namely the introduction of the legume species *Medicago sativa*, *Melilotus suaveolens*, and

Astragalus adsurgens on soil C and N content, and the biomass, activity, and functional diversity of the soil microbial community. Previous studies in this region have shown that the cover and density of *Stipa breviflora*, the target species of restoration, are 2.3 and 18.4 %, respectively, in near native grassland (Li and others 2007), and soil C and N contents are 10.01 ± 0.02 g kg^{-1} and 0.13 ± 0.03 g kg^{-1}, respectively, and soil microbial C and N are 132.34 ± 2.77 mg kg^{-1} and 18.86 ± 1.22 mg kg^{-1} (Li and others 2007; Jia and others 2007); these values can hence be used as targets for the effective functioning of native grassland. The aim of this study, therefore, was to assess the effectiveness of the different legume species in the recovery of soil biological properties and C and N contents in abandoned fields, in order to provide a basis for future decisions on effective grassland restoration programs in this and other regions. We hypothesize that legume species will differentially impact on soil C and N contents, microbial biomass C and N, measured using chloroform-fumigation, microbial activity, measured as basal respiration and metabolic quotient (qCO_2), and substrate utilization rates and functional diversity of soil microbial community, measured using the Biolog EcoPlate method.

Materials and Methods

The Experimental Site

The study was conducted at the Ecological Research Station of Lanzhou University in Yuzhong County, Gansu Province, China (43°26′–44°08′N, 116°04′–117°05′E, 2,400 m above sea level). The field site has a rusty dark loessial soil (Chinese soil taxonomy, Cooperative Research Group on Chinese Soil Taxonomy 1995) or Loess Orthic Entisol (FAO taxonomy, FAO-UNESCO 1988) (sand 12.3 %, silt 66.9 %, clay 20.8 %) with pH values ranging from 8.2 to 8.5 (Shi and others 2003; Wang and others 2008). The area has a semi-arid desert-grassland climate with mean annual precipitation of 360 mm, approximately 80 % of which falls during the 3-month period from June to August. Annual mean temperature is 4.4 °C, the maximum temperature is 19.0 °C (July), and the minimum temperature is −8.0 °C (January). Agriculture in this area is typically based on crops of spring wheat (*Triticum aestivum*), til (*Linsum usitatissinum*), pea (*Pisum sativum*), and potato (*Solanum tuberosum*).

Experimental Design

The effects of sowing the three legume species, *Medicago sativa*, *Astragalus adsurgens*, and *Melilotus suaveolens* (hereafter referred to Med sat, Ast ads, and Mel sua, respectively) on recently abandoned fields, last cropped with til in 2002, were compared with a control site that was harrowed at the same time as the others, but left unseeded for a period after (Control). The recovery effect of legume species on soil properties were also compared with a target soil (Target), another successional control. The seeding treatments were carried out in late April 2003. Four plots for seeding treatments (Med sat, Ast ads, Mel sua, and Control) of 20 × 20 m were assigned in random order in each of three fields. All fields were located within an area of 3 km^2, had the same south facing orientation, and a slope with 20°–25°. All fields were protected against large mammal herbivores (mainly sheep and cattle) with fencing. Each plot was plowed pulling by ox, and legume seeds were broadcasted by hand at the same time. Then the plot was harrowed to incorporate the seeds into soil. Due to differences in seed biomass between the species, standard seed planting densities of 22.5 kg ha^{-1}, 22.5 kg ha^{-1}, and 15 kg ha^{-1} of *Medicago sativa*, *Melilotus suaveolens* and *Astragalus adsurgens,* respectively, were used; therefore, the total number of seeds was same between species (Wang 2000). Before seeding legumes, all the plots were bare for weeds did not germinate before May in this region. The average density of the soil seed bank was (2.328 ± 0.12) × 10^7 seeds ha^{-1}, and there was no significant difference among the plots (ANOVA $P > 0.05$). Soil organic C and N contents before sowing were 4.95 ± 0.11 g kg^{-1} and 0.08 ± 0.01 g kg^{-1}, respectively, across all plots.

Plant Biomass and Soil Physical Characteristics Determination

Ten permanent quadrats of 50 × 50 cm each per plot were set up and randomly placed throughout the plot in spring 2003. The cover and number of plant species in each permanent quadrat were counted at peak standing biomass (5–15 August). Above-ground biomass was sampled by clipping all plants in ten quadrats of 50 × 50 cm^2 situated adjacent to the permanent quadrats that were used for cover assessment. All living vascular plants were sorted into species, dried and weighed. The above-ground biomass was expressed as dry mass m^{-2}.

Soil bulk density was determined on core samples which were taken by driving a metal corer into the soil at the 0–20 cm depth. The soil samples were then oven dried at 105 °C for 24 h and weighed. Bulk density was the weight of dry soil divided by the total soil volume. There were five replications in each plot at 0–20 cm soil depth. Soil moisture (gravimetric water content) in 20 cm was measured by weighting soil samples taken from fields and then oven drying at 105 °C for 24 h and weighting. Soil pH was measured by pH meter with a soil-to-solution ratio of 1:2.5.

Table 1 Plant biomass and soil physical characteristics under different treatments in 2005 and 2007

Years	Treatment	No. of naturally colonizing species	Plant above-ground biomass (g m^{-2})	Soil moisture (%)	Soil bulk density (g cm^{-3})	pH
2005	Med sat	6.8 ± 0.2^{c}	205.5 ± 20.4^{b}	12.93 ± 0.03^{b}	1.28 ± 0.07^{b}	8.36 ± 0.03^{a}
	Ast ads	6.6 ± 0.4^{bc}	360.0 ± 28.8^{c}	10.48 ± 0.02^{a}	1.23 ± 0.06^{ab}	8.78 ± 0.01^{b}
	Mel sua	6.0 ± 0.4^{b}	244.0 ± 19.2^{b}	13.07 ± 0.03^{b}	1.20 ± 0.03^{a}	8.64 ± 0.01^{ab}
	Control	5.6 ± 0.2^{a}	82.9 ± 7.8^{a}	14.16 ± 0.03^{c}	1.33 ± 0.05^{b}	8.80 ± 0.03^{b}
2007	Med sat	5.4 ± 0.4^{b}	133.6 ± 15.4^{d}	9.28 ± 0.00^{b}	1.23 ± 0.09^{a}	8.39 ± 0.03^{a}
	Ast ads	7.0 ± 0.3^{c}	93.2 ± 8.8^{b}	6.02 ± 0.02^{a}	1.21 ± 0.01^{a}	8.56 ± 0.01^{b}
	Mel sua	7.0 ± 0.6^{c}	111.5 ± 10.2^{c}	10.53 ± 0.00^{b}	1.21 ± 0.03^{a}	8.43 ± 0.01^{a}
	Control	4.0 ± 0.4^{a}	43.6 ± 3.8^{a}	12.31 ± 0.03^{c}	1.31 ± 0.05^{b}	8.48 ± 0.03^{ab}

Values are means and SE and those with the *same letter* are not significantly different at the $P < 0.05$ levels

Med sat = *Medicago sativa*, Ast ads = *Astragalus adsurgens*, Mel sua = *Melilotus suaveolens*

Plant above-ground biomass and soil physical characteristics are presented in Table 1.

Soil Organic Carbon and Total Nitrogen, Microbial C and N Determinations

After above-ground biomass sampling, three quadrats in each plot were randomly selected to collect soil samples in years of 2003, 2005, and 2007 at peak standing biomass (5–15 August). Five cores (5 cm in diameter and 20 cm in depth) of 0–20 cm surface soil were taken randomly in each quadrat and mixed completely to get a composite fresh sample for each quadrat, for a total of three samples per plot and nine samples per treatment each year. After removing large plant materials and fine roots, each sample was divided into two parts. One part of each soil sample was air-dried for the estimation of soil physicochemical parameters and the other part was used to assess biological properties. Specifically, soil C and N in microbial biomass was measured in 2005 and 2007. Basal respiration and metabolic quotient, as well as bacterial functional diversity of the microbial community were measured in 2007. This later part was sieved through a 2 mm screen and adjusted to 50 % of its water holding capacity and then incubated at 25 °C for 2 weeks to permit uniform rewetting and to stabilize the microbial activity after the initial disturbances.

For each sample, 0.5 g dry soil sample was used to measure soil organic C (TOC) using the dichromate oxidation method (Kalembasa and Jenkinson 1973) and 1 g dry soil sample was used to measure soil total N (TN) by the Kjeldahl method (Jackson 1958). Microbial C and N were determined by using the chloroform fumigation extraction method (Brookes and others 1985). Three 25 g sub-samples for each sample were fumigated with alcohol-free $CHCl_3$ for 24 h at 25 °C. These samples were then extracted by adding 100 ml of 0.5 M K_2SO_4, shaking for 60 min and filtered through Whatman No. 2 paper. Three non-fumigated soil samples for each sample were processed in the same manner. Microbial biomass C (MBC) was estimated as the difference between fumigated and nonfumigated samples divided by the K_2SO_4 extract efficiency factor for microbial C ($Kc = 0.38$, Vance and others 1987). Microbial biomass nitrogen (MBN) was calculated by dividing the total N difference between fumigated and non-fumigated samples with K_2SO_4 extract efficiency factor for microbial N ($K = 0.54$, Brookes and others 1985).

Basal Respiration and Metabolic Quotient

Basal respiration was determined by quantifying the carbon dioxide (CO_2) released in the process of microbial respiration during incubation at 28 °C for 24 h. This was done by placing 25 g soil samples into 3.0 l capacity glass containers with hermetic lids, together with a smaller flask containing 20 ml 1 M NaOH to capture the released CO_2. The container was tightly sealed. CO_2 was determined by titration with 0.5 M HCl, after precipitation of the barium carbonate formed by adding barium chloride ($BaCl_2$) aqueous solution to the NaOH solution, utilizing phenolphthalein diluted into 100 ml ethanol (60 %,v/v) as an indicator. The metabolic quotient (qCO_2) was calculated as the ratio of basal respiration to microbial biomass C (Anderson and Domsch 1990).

Bacterial Functional Diversity in Biolog EcoPlate

EcoPlate was used to assess the functional diversity of the microbial community, measured by Biolog EcoPlate™ system (Garland and Mills 1991; Insam 1997) which is a rapid, community-level physiological profile (CLPPs) approach for assessing patterns of sole C source utilization by mixed microbial samples (Garland 1996; Gomez and others 2004; Nautiyal and others 2008). Each Biolog

EcoPlate has 31 different carbon substrates with triplicates providing a metabolic pattern of the microbial community. Ninety-six well microplates were inoculated with 150 μl of the 10^{-2} suspension per well. This dilution was chosen in order to minimize the influence of soil-borne C sources and soil particles on the color development, which is indicative of carbon-source utilization. Plates were incubated at 28 °C for 168 h in the dark. The rate of utilization is indicated by the reduction of tetrazolium, a redox indicator dye, which changes from colorless to purple. Data were recorded at 12 h intervals for 7 days at 590 nm with Vamax reader (Microlog ReL 3.5). Microbial activity in each microplate, expressed as average well color development (AWCD), was determined as described by Garland (1996).

AWCD and Shannon–Wiener diversity index in Biolog EcoPlate were calculated using data of Biolog EcoPlate collected during 0–168 h and at 168 h, respectively, as described by Nautiyal (2009) and Staddon and others (1997).

$AWCD = \sum(C–R)/31$, C is sum of optical densities of 31 wells in a plate, R is optical density of control well in the same plate.

Shannon–Wiener diversity $H = -\Sigma P_i \ln P_i$, of which P_i is proportional color development of the ith well over total color development of all wells of a plate.

Statistical Analysis

Data were analyzed using SPSS 16.0 and Origin 7.0. Spearmen's correlation coefficients (r) were calculated and ANOVAs were used for individual treatment comparisons at $\alpha = 0.05$, with separation of means by LSD. Principal component analysis (PCA) was performed on data divided by the AWCD of 168 h as described by Merkl and Schultze-Kraft (2006), Chen and others (2011) to discriminate the carbon (C) utilization patterns of soil microbial communities.

Results

Soil TOC and TN, MBC, and MBN

The introduction of legumes significantly affected TOC ($F_{0.05\ (3,107)} = 5.273$, $P = 0.012$) and TN ($F_{0.05\ (3,107)} = 2.843$, $P = 0.042$), and as time progressed, both these measures increased across all treatments (Fig. 1). TOC was significantly greater in soil planted with *Medicago sativa* than in the control, while TOC in soil planted with *Astragalus adsurgens* was similar to that in the control, except in 2005 when this measure was greater than in the control. However, TOC in soil of *Melilotus suaveolens* was significantly lower than with the other two legumes treatments or the control, except that in 2005. Soil TN in the *Medicago sativa* treatment was significantly higher than in other two legumes treatments and also greater than in the control in each year. TN in soil of *Melilotus suaveolens* treatment was lower than that in the control in 2005 and 2007, but this difference was only significant in 2007. Compared to the target values, soil TOC under *Medicago*

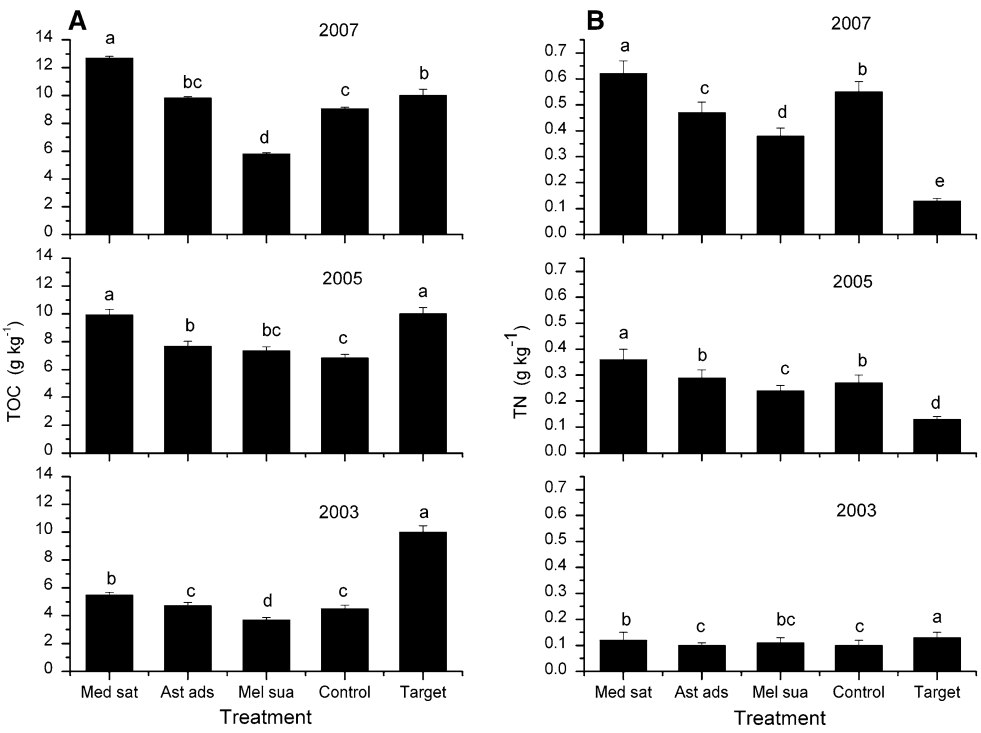

Fig. 1 Soil carbon (**A**) and nitrogen (**B**) properties under different treatments in different years. Med sat = *Medicago sativa*; Ast ads = *Astragalus adsurgens*; Mel sua = *Melilotus suaveolens*; *TOC* total organic carbon; *TN* total nitrogen. Values are means and SE ($n = 9$) and those with the same letter are not significantly different at the $P < 0.05$ levels

sativa in 2005, and *Medicago sativa* and *Astragalus adsurgens* in 2007, and TN under all three legumes treatments in 2005 and 2007, was higher (Fig. 1).

Legume species significantly affected soil microbial biomass C ($F_{0.05\ (3,71)} = 4.420$, $P = 0.0008$) and N ($F_{0.05\ (3,71)} = 2.979$, $P = 0.016$). Soil MBC and MBN in the *Medicago sativa* and *Astragalus adsurgens* treatments were significantly greater than that in the control, although they were greatest in soil of *Medicago sativa* (Fig. 2). MBC and MBN in soil of *Melilotus suaveolens* was significantly greater than that in the control and *Astragalus adsurgens* treatment in 2005, but significantly lower than in the control and *Astragalus adsurgens* treatment in 2007. Soil MBC under the *Medicago sativa* and *Astragalus adsurgens* treatments in 2005 and 2007, and the *Melilotus suaveolens* treatment in 2005, were greater than the target soil values, while under the *Medicago sativa* treatment, in both years, MBN was greater than the target values (Fig. 2). Microbial biomass C and N were positively related to soil total N ($r = 0.772$, $P < 0.01$; $r = 0.863$, $P < 0.05$, respectively), but not TOC (Table 2).

The ratio of MBC:MBN was greater in all legume treatments relative to the control, although this response was greatest for *Medicago sativa* and *Melilotus suaveolens* in 2005, and *Astragalus adsurgens* in 2007 (Fig. 2) ($F_{0.05\ (3,71)} = 3.535$,

Table 2 Coefficients of correlation between the microbial and chemical properties of soils

	TOC	TN	MBC	MBN
TOC	1.000			
TN	0.826**	1.000		
MBC	0.48	0.772**	1.000	
MBN	0.629	0.863*	0.573	1.000

TN total nitrogen; *TOC* total organic carbon; *MBC* microbial biomass carbon

* $P < 0.05$

** $P < 0.01$

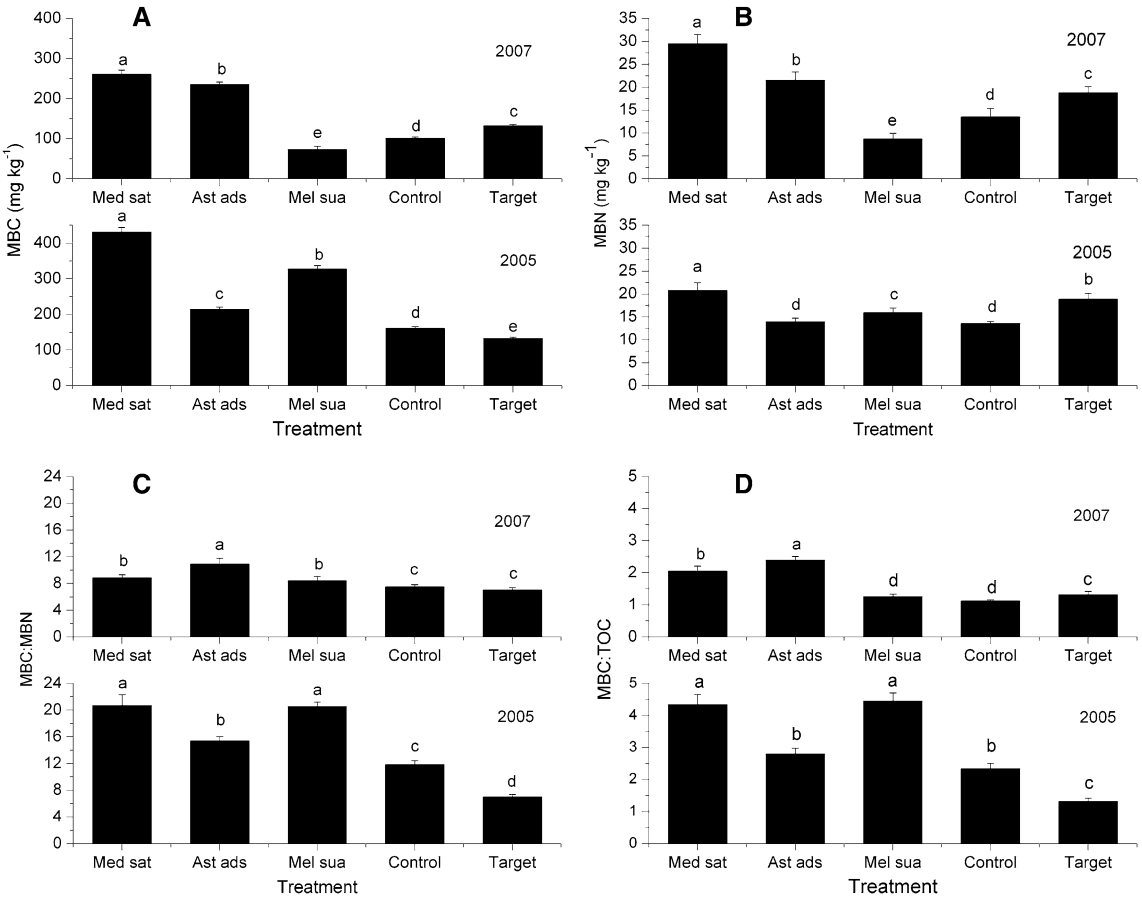

Fig. 2 Soil microbial biomass carbon (**A**) and nitrogen (**B**) and ratios of MBC:MBN (**C**) and MBC:TOC (**D**). Med sat = *Medicago sativa*; Ast ads = *Astragalus adsurgens*; *Mel sua* = *Melilotus suaveolens*; *TOC* total organic carbon; *MBC* microbial biomass carbon; *MBN* microbial biomass nitrogen; *MBC:MBN* ratio of microbial biomass carbon and nitrogen; *MBC:TOC* ratio of microbial biomass carbon and total organic carbon. Values are means and SE ($n = 9$) and those with the *same letter* are not significantly different at the $P < 0.05$ levels

$P = 0.021$). The value increased to 20.70 in *Medicago sativa* treatment compared to 11.86 in the control in 2005, while 10.92 in *Astragalus adsurgens* treatment compared to 7.48 in the control in 2007. The ratio of MBC:TOC was greatest in soil of the *Medicago sativa* and *Astragalus adsurgens* treatments relative to the control in 2005, but there was no difference in this measure for soil of the *Astragalus adsurgens* treatment and the control. However, the ratio of MBC:TOC decreased in 2007, and it was greatest in *Astragalus adsurgens,* followed by the *Medicago sativa* treatment. There was no difference in this measure for soil of the *Melilotus suaveolens* treatment and the control.

Basal Respiration and Metabolic Quotient

Basal respiration ranged from 8.46 to 11.76 mg $C{-}CO_2$ kg^{-1} soil h^{-1} across legume treatments, which was significantly higher than that of the control ($F_{0.05\ (3,35)} = 7.315$, $P = 0.0004$), although there was no significant difference among legumes treatments except *Melilotus suaveolens* and *Astragalus adsurgens* (Table 3). Basal respiration was highest in soils of the *Medicago sativa* and *Astragalus adsurgens* treatments. In general, qCO_2 was significantly greater in soils of the legume treatments relative to the control ($F_{0.05(3,35)} = 3.535$, $P = 0.021$) (Table 3) and there were significant differences across all treatments except between *Medicago sativa* and *Astragalus adsurgens.*

AWCD and Functional Diversity of Soil Microbial Community

In general, during 168 h period of incubation, soil microbial communities under legumes had higher activities than the control (Fig. 3), although this measure was greatest under *Astragalus adsurgens*, followed by *Melilotus suaveolens*, *Medicago sativa* and then the control. At the early incubation time (60 h), there was no significant difference among all treatments, except *Astragalus adsurgens* and control. However, after 60 h incubation, there was significant difference across all treatments ($F_{0.05\ (3,11)} = 9.274$, $P = 0.009$).

PCA of sole C source utilization patterns for soil microbial community, based on the OD of Biolog EcoPlate at 168 h incubation, showed that principal component 1 (PC1) explained 37.47 % of variance, which is mainly the carboxylic acid carbon source (except α-D-lactose and D-cellubiose). Principle component 2 (PC2) explained 17.86 % of variance, which is mainly the carbohydrate carbon source (except 1-phosphate glucose and glycine L-glutamate) (Table 4). The PCA results showed that the C substrate utilizing profiles were separated into two distinct groups: legume treatments and the control (Fig. 4). The main substrates utilized by microbes under legumes were L-asparagine, D-galactoic acid γ-lactone, D-gluconic acid, L-serine, and glycyl L-glutamine while those under control were

Table 3 Basal respiration ($C{-}CO_2$ mg kg^{-1} h^{-1}) and metabolic quotient (qCO_2) under different treatments

Treatment	Basal respiration ($C{-}CO_2$ mg kg^{-1} h^{-1})	Metabolic quotient (qCO_2)
Med sat	10.06 ± 0.90^{ab}	0.038 ± 0.002^{b}
Ast ads	8.46 ± 0.63^{b}	0.115 ± 0.009^{a}
Mel sua	11.76 ± 0.70^{a}	0.037 ± 0.002^{b}
Control	1.85 ± 0.04^{c}	0.018 ± 0.001^{c}

Values are means and SE and those with the *same letter* are not significantly different at the $P < 0.05$ levels

Med sat = *Medicago sativa*, Ast ads = *Astragalus adsurgens*, Mel sua = *Melilotus suaveolens*

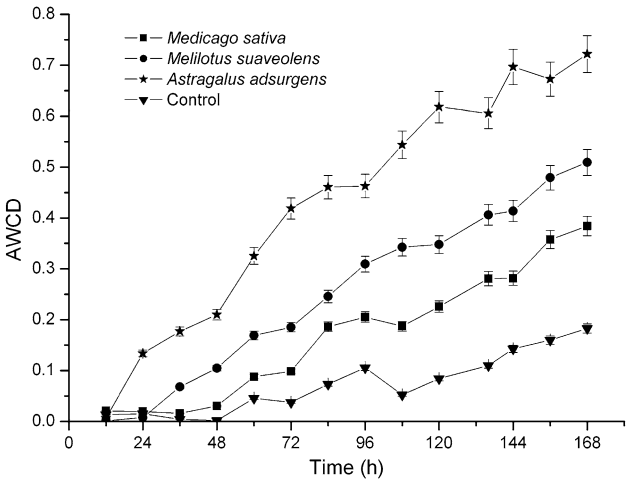

Fig. 3 Average well color development (AWCD) (means and SE ($n = 3$)) of soil microbial community in different treatments during incubation

Table 4 Main carbon with high correlation coefficients for PC1 and PC2

PC1	*r*	PC2	*r*
α-Ketobutyric acid	0.968	α-Cyclodextrin	0.765
Phenylethylamine	0.912	1-Phosphate glucose	0.764
Itaconic acid	0.896	β-Methyl glucoside	0.754
D-Xylose	0.876	Tween 40	0.803
L-Threonine	0.876	Glycine L-glutamate	0.674
γ-Hydroxybutyrate	0.876	Glycoside sugar	0.663
N-acetyl-D-glucosamine	0.806	Pyruvic acid methyl ester	0.657
α-D-Lactose	0.745	Tween 80	0.653
D-Cellubiose	0.720	D-Glucuronides	0.634
2-Hydroxybenzoic acid	0.653		
L-Serine	0.650		

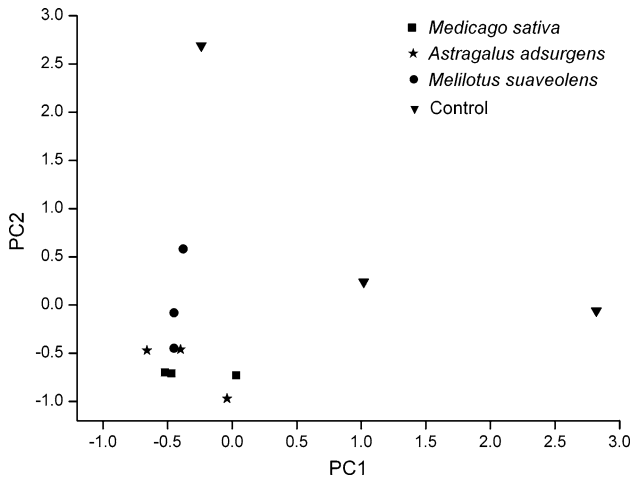

Fig. 4 PCA of soil microbial communities under different treatments

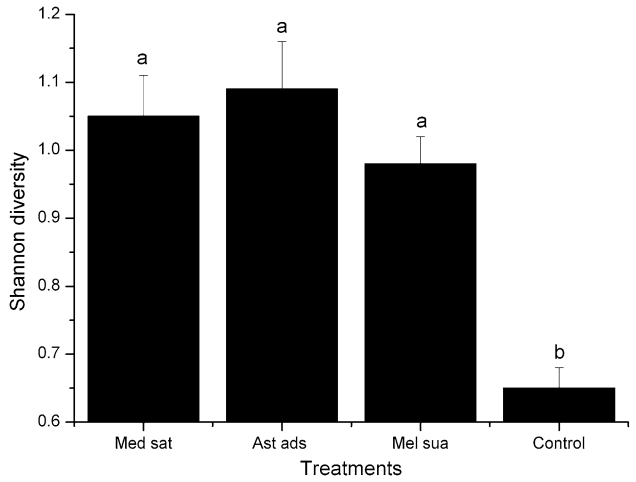

Fig. 5 Diversity index of soil microbial communities (means and SE ($n = 3$)) calculated using data of Biolog EcoPlate after 168 h of incubation. Med sat = *Medicago sativa*; Ast ads = *Astragalus adsurgens*; Mel sua = *Melilotus suaveolens*. Values with the *same letter* are not significantly different at the $P < 0.05$ level

Tween 80, Tween 40, pyruvic acid methyl ester, L-arginine, D-xylose, L-phenylalanine, α-ketobutyric acid, phenylethylamine, D, L-α-glycerophosphate, and 4-hydroxybenzoic acid. The metabolic Shannon diversity of soil microbial community in Biolog EcoPlate under legumes were significantly higher than that of control ($F_{0.05\,(3,\,11)} = 6.969, P = 0.013$), although there was no significant difference among legumes treatments. The highest Shannon diversity index was in the soil of *Astragalus adsurgens* (Fig. 5).

Discussion

Our results show that the effects of legumes on soil microbial communities and soil C and N contents are both species and time dependant. After 3–5 years since their introduction, the perennial legumes *Medicago sativa* and *Astragalus adsurgens* positively affected soil microbial biomass and soil C content. In contrast, after 3 years, the annual or biennial legume *Melilotus suaveolens* had neutral or negative effects on soil C and N, and positively affected soil microbial biomass, while this species had negative effects on these measures after 5 years. Soil under *Medicago sativa* had the highest soil microbial biomass and C and N content (Figs. 1, 2). Such variation in the effect of legume species on soil microbial biomass and soil C and N contents could be due to differences in the productivity and nutrient content of the three legumes (Yang and others 2005), as well as differences in life history and other growth characteristics (Cheng and others 2004; Cheng and others 2005). For instance, *Astragalus adsurgens* had the highest above-ground biomass and highest total N and phosphorus (P) content of shoot and root, while *Melilotus suaveolens* had the lowest total N and P content (Yang and others 2005).

In both 2005 and 2007 in the *Medicago sativa* treatment, and in 2007 for the *Astragalus adsurgens* treatment, soil C contents were greater than the target value derived from native grassland, the desired end-point of restoration. However, soil N contents in 2005 exceeded the target for this measure in all three legume treatments, although soil N contents were greatest for *Medicago sativa* (Fig. 1). These results indicate that soil C contents could be restored in 3 years through the introduction of *Medicago sativa*, and in 5 years through the introduction of *Astragalus adsurgens,* whereas soil N can be restored within 3 years with all legumes. Higher soil MBC in the *Medicago sativa* and *Astragalus adsurgens* treatments in both 2005 and 2007 and higher MBN in the *Medicago sativa* treatment in 2005 and in the *Medicago sativa* and *Astragalus adsurgens* treatments in 2007 (Fig. 2) indicated MBC and MBN could be recovered after 3–5 years since their introduction. Overall, these results indicate that soil C and N could be effectively restored by introducing *Medicago sativa* and *Astragalus adsurgens* into early abandoned fields, whereas it could be retarded by introducing *Melilotus suaveolens* as evidenced by lower TOC, TN in this treatment relative to control in 3 years and lower MBC and MBN in 2007 (Figs. 1, 2).

The introduction of the legumes *Medicago sativa* and *Astragalus adsurgens* increased soil microbial biomass, the ratio of MBC:MBN, basal respiration and the metabolic quotient (qCO_2), and the activity and functional diversity of the microbial community, measured using the Biolog EcoPlate method. In contrast, *Melilotus suaveolens* reduced soil microbial biomass C and N after 5 years (Figs. 2, 3, 4, 5). The positive effect of the two legumes on soil microbial communities is likely due to the promotion of plant biomass (Table 1) (Li and others 2007), and hence the amount of N-rich organic matter entering the soil, as well as nitrogen availability (Wang and Hu 2001), which could cause

microbial populations to flourish. The high ratio of MBC:MBN generally, which ranged from 7.48 to 10.92, might suggest that the microbial biomass contains a higher proportion of fungi in soil of these abandoned-fields, and that all legume species increase the proportion of fungi biomass in soil microbial community (Jenkinson 1976; Pichtel and Hayes 1990; Paul and Clark 1996; Kara and Bolat 2008).

The negative effect of *Melilotus suaveolens* on soil microbial biomass after 5 years was likely due to reduced soil moisture (Table 1), given that this species is known to be very water demanding and its growth slows down and its biomass decreases due to deficiency of soil moisture on *Astragalus adsurgens* grasslands from the fifth year (Cheng and others 2004), as well as the previously shown inhibitive effect of this legume on natural colonizers (Li and others 2008). The notion that this legume species reduced soil moisture is supported by the fact that soil water contents were least under *Melilotus suaveolens* than under the other two legumes, and in the surface soil (0–20 cm) or the succession control during the growing season (Jia 2007). The inhibitive effect of the three legumes on weeds disappeared after 3 years, although the strongest effect in the first 2 years appeared in the *Melilotus suaveolens*-sown treatment (Li and others 2008). Although *Melilotus suaveolens* gradually disappeared from the community, its effect on soil desiccation remained for at least 3–5 years (Table 1). Combined, the decrease in soil water content and input of organic matter likely inhibited the growth of soil microorganisms; a view that is supported by the knowledge soil microbial biomass in semiarid temperate steppe is strongly regulated by these factors (Liu and others 2009). These legume species not only increased soil organic matter (except *Melilotus suaveolens*), but also affected its quality and availability of organic matter for microorganisms (Insam and Domsch 1988; Anderson and Domsch 1990; Wang and others 2003) as evidenced by the increase in the MBC:TOC (Fig. 2) and thereafter significantly affected the number and activity of soil microorganisms (Powlson and others 1987; VA'Squez_Murrieta and others 2007). More active microbial communities (Figs. 3, 5) of lower biomass in the *Melilotus suaveolens* treatment (Fig. 2) could explain the greater qCO_2 in this treatment, although greater water limitation in this treatment might also play a role (Wardle and Ghani 1995).

Past studies have shown that there is a positive relationship between soil organic C and microbial biomass (Guggenberger and Zech 1999; Piao and others 2001; Liu and others 2010). However, in our study, MBC and MBN were positively related to soil total N, but not organic C, which was in accordance with a previous study of Jia and others (2007) in this region. Differences in AWCD could result from differences in the biomass and nutrient content of the legumes, given that *Astragalus adsurgens* had the highest biomass and total N and P content (Yang and others 2005). Previous research has shown that the differences in AWCD are in part due to lower microbial bio-mass (Garland 1996; Grayston and others 2004). In our study, AWCD values were correlated with microbial biomass ($r = 0.771$, $P < 0.05$, data not shown), which was consistent with other report (Bååth 1998), and might be due to variations in both the quantity and quality of organic matter in soil. PCA analysis of microbial communities revealed distinct different patterns of C utilization for the legumes treatments and the control. The pattern of C utilization in natural succession was clearly separated from those in legumes treatments (Fig. 4), which was generally consistent with AWCD (Fig. 3). Sugars and carboxylic acids were the main C sources utilized by the soil microbial community in the legumes treatments. This could result from differences in the composition of root exudates across legume species (Nannipieri and others 2008), and root exudates from legumes containing rapid utilization substrates by bacteria, such as sugars, amino acids and carboxylic acids (Gaworzewska and Carlile 1982). It could also be due to variations in the quality of litter inputs from roots and shoots among legume species.

In general, the impact on the soil microbial community and concentrations of C and N of introducing legumes into abandoned fields depended on time-scale and were species-specific. Soil C and N could be effectively restored by introducing *Medicago sativa* and *Astragalus adsurgens* into early abandoned fields. However soil C and N was retarded after 3–5 years, as was the biomass of microbes after 5 years following the introduction of *Melilotus suaveolens*. Overall, our results show that the restoration practice of adding legumes significantly affects soil C and N contents and the abundance, activity and functional diversity of the microbial community, albeit in a species specific way. Therefore, we propose that certain legume species could be used to accelerate ecological restoration of degraded soils, and hence assist in the protection and preservation of the environment.

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References

Anderson TH, Domsch KH (1990) Application of ecophysiological quotients (qCO_2 and qD) on microbial biomass from soils of different cropping histories. Soil Biol Biochem 22:251–255

Anderson TH, Domsch KH (2010) Soil microbial biomass: the eco-physiological approach. Soil Biol Biochem 42:2039–2043

Bååth E (1998) Growth rates of bacterial communities in soils at varying pH: a comparison of the thymidine and leucine incorporation techniques. Microbial Ecology 36:316–327

Bardgett RD (2005) The biology of soil: a community and ecosystem approach. Oxford University Press, Oxford

Bardgett RD, Freeman C, Ostle NJ (2008) Microbial contributions to climate change through carbon cycle feedbacks. ISME J 2:805–814

Bending GD, Turner MK, Rayns F, Marx MC, Wood M (2004) Microbial and biochemical soil quality indicators and their potential for differentiating areas under contrasting agricultural management regimes. Soil Biol Biochem 36:1785–1792

Brookes PC (1995) The use of microbial parameters in monitoring soil pollution by heavy metals. Biol Fertil Soils 19:269–279

Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol Biochem 17:837–842

Catt JA (2001) The agricultural importance of loess. Earth Sci Rev 54:213–229

Chen SN, Gu J, Gao H, Qin QJ (2011) Effect of microbial fertilizer on microbial activity and microbial community diversity in the rhizosphere of wheat growing on the Loess Plateau. Afr J Microbiol Res 5:137–143

Cheng JM, Wan HE, Wang J, Yong SP (2004) Over depletion and recovery of soil moisture on *Astragalus adsurgens* grasslands in the loess hilly-gully region. Acta Ecologica Sinica 24:2979–2983

Cheng JM, Wan HE, Wang J (2005) Alfalfa growth and its relation with soil water status in loess hilly and gully region. Chin J Appl Ecol 16:435–438

Chodak M, Pietrzykowski M, Niklin′ska M (2009) Development of microbial properties in a chronosequence of sandy mine soils. Appl Soil Ecol 41:259–268

Cooperative Research Group on Chinese Soil Taxonomy (1995) Chinese soil taxonomy. Agricultural Science and Technology Press, Beijing

De Deyn GB, Quirk H, Zou Y, Oakley S, Ostle NJ, Bardgett RD (2009) Vegetation composition promotes carbon and nitrogen storage in model grassland communities of contrasting soil fertility. J Ecol 97:864–875

De Deyn GB, Shiel RS, Ostle NJ, McNamara NP, Oakley S, Young L, Freeman C, Fenner N, Quirk H, Bardgett RD (2011) Additional carbon sequestration benefits of grassland diversity restoration. J Appl Ecol 48:600–608

De Vries FT, Liiri M, BjÃ¸rnlund L, Bowker MA, Christensen S, Setala HM, Bardgett RD (2012) Land use alters the resistance and resilience of soil food webs to drought. Nature Climate Change 2:276–280

Degens FBP, Schipper LA, Sparling GP, Duncan LC (2001) Is the microbial community in soil with reduced catabolic diversity less resistant to stress or disturbance? Soil Biol Biochem 33:1143–1153

FAO-UNESCO (1988) Soil map of the world. World soil resources report 60. FAO, Rome

Fornara DA, Tilmanm D (2008) Plant functional composition influences rates of soil carbon and nitrogen accumulation. J Ecol 96:314–322

Fu LG (2001) Higher plants of China, vol 7. Qingdao Publishing House, Qingdao

Gan ZM, Sang GS (2002) Discussion on the cultivated land reduction and forest and grass on Loess Plateau. J Arid Land Resour Environ 16:62–65

Garland JL (1996) Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. Soil Biol Biochem 28:213–221

Garland JL, Mills AL (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl Environ Microbiol 7:2351–2359

Gaworzewska ET, Carlile MJ (1982) Positive chemotaxis of *Rhizobium leguminosarum* and other bacteria towards root exudates from legumes and other plants. J Gen Microbiol 128:1179–1188

Gomez E, Garland J, Conti M (2004) Reproducibility in the response of soil bacterial community-level physiological profiles from a land use intensification gradient. Appl Soil Ecol 26:21–30

Grayston SJ, Campbell CD, Brdgett RD, Mawdsley JL, Clegg CD, Ritz K, Griffiths BS, Rodwell JS, Edwards SJ, Davies WJ, Elston DJ, Millard P (2004) Assessing shifts in microbial community structure across a range of grasslands of differing management intensity using CLPP, PLFA and community DNA techniques. Appl Soil Ecol 25:63–84

Guggenberger G, Zech W (1999) Soil organic matter composition under primary forest, pasture, and secondary forest succession, Región Huetar Norte, Costa Rica. Forest Ecol Manag 124:93–104

Hooper DU, Vitousek PM (1998) Effects of plant composition and diversity on nutrient cycling. Ecol Monogr 68:121–149

Hopkins A, Wilkins RJ (2006) Temperate grassland: key developments in the last century and future perspectives. J Agric Sci 144:503–523

Insam H (1997) A new set of substrates proposed for community characterization in environmental samples. In: Insam H, Rangger A (eds) Microbial communities: functional versus structural approaches. Springer, Berlin, pp 259–260

Insam H, Domsch KH (1988) Relationship between soil organic carbon and microbial biomass on chronosequences of reclamation sites. Microb Ecol 15:177–188

Jackson ML (1958) Soil chemical analysis. Prentice-Hall, New Delhi

Jenkinson DS (1976) The effects of biocidal treatments on metabolism in soil-IV. The decomposition of fumigated organisms in soil. Soil Biol Biochem 8:203–208

Jia JJ (2007) Effects of introducing legume species to abandoned-fields on abiotic properties and microbial biomass. Dissertation, Lanzhou University

Jia JJ, Li JH, Wang G, Li WJ (2007) Effects of the introduction of legume species on soil nutrients and microbial biomass of abandoned-fields. J Lanzhou Univ Nat Sci 43:33–37

Kalembasa SJ, Jenkinson DS (1973) A comparative study of titrimetric and gravimetric methods for the determination of organic carbon in soil. J Sci Food Agric 24:1085–1109

Kara O, Bolat I (2008) Soil microbial biomass C and N changes in relation to forest conversion in the northwestern turkey. Land Degrad Dev 19:421–428

Kardol P, Wardle DA (2010) How understanding aboveground-belowground linkages can assist restoration ecology. Trends Ecol Evol 25:670–679

Kardol P, Bezemer TM, van der Putten WH (2006) Temporal variation in plant–soil feedback controls succession. Ecol Lett 9:1080–1088

Landi F, Valori J, Ascher J, Renella G, Falchini L, Nannipieri P (2006) Root exudates effects on the bacterial communities, CO_2 evolution, nitrogen transformations and ATP content of rhizosphere and bulk soils. Soil Biol Biochem 38:509–516

Li JH, Fang XW, Jia JJ, Wang G (2007) Effect of legume species introduction to early abandoned field on vegetation development. Plant Ecol 191:1–9

Li JH, Xu DH, Wang G (2008) Weed inhibition by sowing legume species in early succession of abandoned fields on Loess Plateau, China. Acta Oecologica 33:10–14

Liu DS, Ding ML (2004) The Loess Plateau, origin of agriculture and water and soil conservation. Earthquake Press, Beijing

Liu Y, Han S, Hu Y, Dai G (2005) Effects of soil temperature and humidity on soil respiration rate under *Pinus sylvestriformis* forest. Chin J Appl Ecol 16:1581–1585

Liu WX, Zhang Z, Wan SQ (2009) Predominant role of water in regulating soil and microbial respiration and their responses to climate change in a semiarid grassland. Glob Change Biol 15:184–195

Liu WX, Xu WH, Hong JP, Wan SQ (2010) Interannual variability of soil microbial biomass and respiration in responses to topography, annual burning and N addition in a semiarid temperate steppe. Geoderma 158:259–267

Merkl N, Schultze-Kraft R (2006) Influence of tropical grass *Brachiaria brizantha* (Hochst. ex A. Rich.) stapf on bacterial community structure in petroleum contaminated soils. Int J Soil Sci 1:108–117

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G, Valori F (2008) Effects of root exudates in microbial diversity and activity in rhizosphere soils. In: Nautiyal CS, Dion P (eds) Molecular mechanisms of plant and microbe coexistence. Springer, Berlin, pp 339–368

Nautiyal CS (2009) Self-purificatory Ganga water facilitates death of pathogenic *Escherichia coli* 157:H7. Curr Microbiol 58:25–29

Nautiyal CS, Govindarajan R, Lavania M, Pushpangadan P (2008) Novel mechanism of modulating natural antioxidants in functional foods: involvement of plant growth promoting rhizobacteria NRRL B-30488. J Agric Food Chem 56:4474–4481

Ohashi H, Tateishi Y (1984) The science reports of the Tohoku University. Fourth Series. Biology 38:320

Paul EA, Clark FE (1996) Soil Microbiology and Biochemistry. Academic Press, San Diego

Piao HC, Liu GS, Wu YY, Xu WB (2001) Relationships of soil microbial biomass carbon and organic carbon with environmental parameters in mountainous soils of southwest China. Biol Fertil Soils 33:347–350

Pichtel JR, Hayes JM (1990) Influence of fly ash on soil microbial activity and populations. J Environ Qual 19:593–597

Powlson DS, Brooks PC, Christensen BT (1987) Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. Soil Biol Biochem 19:159–164

Rochon JJ, Doyle CJ, Greef JM (2004) Grazing legumes in Europe: a review of their status, management, benefits, research needs and future prospects. Grass Forage Sci 59:197–214

Saviozzi A, Levi-Minzi R, Cardelli R, Riffaldi R (2001) A comparison of soil quality in adjacent cultivated, forest and native grassland soils. Plant Soil 233:251–259

Shi ZY, Liu WZ, Guo SL, Li FM (2003) Moisture properties in soil profiles and their relation to landform at Zhonglianchuan small watershed. Agric Res Arid Areas 21:101–104

Soussana JF, Loiseau P, Vuichard N, Ceschia E, Balesdent J, Chevallier T, Arrouays D (2004) Carbon cycling and sequestration opportunities in temperate grasslands. Soil Use Manag 20:219–230

Sparling GP (1992) Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter. Aust J Soil Res 30:195–207

Staddon WJ, Duchesne LC, Trevors JT (1997) Microbial diversity and community structure of post-disturbance forest soils as determined by sole-carbon-source utilization patterns. Microb Ecol 34:125–130

VA′ Squez-Murrieta MS, Govaerts B, Dendooven L (2007) Microbial biomass C measurements in soil of the central highlands of Mexico. Appl Soil Ecol 35:432–440

Van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707

Wang ZL (2000) Cultivation technology of legumes *Astragalus adsurgens*, *Melilotus suaveolens*, *and Medicago sativa*. Forest Technol Hebei Province, pp 65–69

Wang WW, Hu ZH (2001) Characteristic of symbiotic nitrogen fixation of main soil and water conservation plants in Gansu-Ningxia region of Loess Plateau. Bull Soil Water Conserv 21:30–33

Wang WJ, Dalal RC, Moody WP, Smith CJ (2003) Relationships of soil respiration to microbial biomass, substrate availability and clay content. Soil Biol Biochem 35:273–284

Wang XL, Sun GJ, Jia Y, Li FM, Xu JZ (2008) Crop yield and soil water restoration on 9-year-old alfalfa pasture in the semiarid Loess Plateau of China. Agric Water Manag 95:190–198

Wardle DA, Ghani A (1995) A critique of the microbial metabolic quotient (qCO_2) as a bioindicator of disturbance and ecosystem development. Soil Biol Biochem 27:1601–1610

Xue ZD, Hou QC, Han RL, Wang SQ (2002) Trails and research on ecological restoration by *Sophora viciifolia* in Gullied Rolling Loess Region. J Northwest Forest Univ 17:26–29

Yan F, McBratney AB, Copeland L (2000) Functional substrate biodiversity of cultivated and uncultivated A horizons of vertisols in NW New South Wales. Geoderma 96:321–343

Yang XH, Wang BF, Jiang ZP (2005) Biomass and nutrient concentrations of three green manure leguminous plants and their effects on soil nutrients at northeastern edge of Ulanbuhe Desert. Chin J Ecol 24:1134–1138

Zeng DH, Hu YL, Chang SX, Fan ZP (2009) Land cover change effects on soil chemical and biological properties after planting Mongolian pine (*Pinus sylvestris* var. mongolica) in sandy lands in Keerqin, northeastern China. Plant Soil 317:121–133

Zou HY, Chen JM, Zhou L, Hongo A (1998) Natural recoverage succession and regulation of the Prairie vegetation on the Loess Plateau. Res Soil Water Conserv 5:126–138