

# Benefits of flavonoids and straw mulch application on soil microbial activity in pea rhizosphere

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**Abstract** The importance of flavonoids in rhizosphere–legumes symbiosis has been recognized as critical. However, the limited data are available about their impact on soil microbial communities. In rhizosphere, it remains unclear whether flavonoids, mulch or their joint effects influence on soil microbial diversity and enzymes activity. Therefore, in this study, the effects of flavonoids and straw mulching on soil microbial functional diversity, fungi abundance and enzymes activity (dehydrogenases, protease, acid phosphomonoesterase) in pea rhizosphere were evaluated. The field study was conducted in Lublin, Poland (51°15'N, 22°35'E), on a Haplic Luvisol. Flavonoids were applied on pea seeds and after sowing soil surface was covered with straw mulch. In soil rhizosphere sampled three times during the vegetative period of pea were determined: dehydrogenases, protease and acid phosphomonoesterase activities, metabolic potential of soil bacteria, microbes number and predominant fungal species. The results showed that dehydrogenases and protease activities were significantly increased with time during pea growing season. Significant increase in dehydrogenases activity was observed after flavonoids and mulch influence. There was no impact of studied factors on acid phosphomonoesterase. The effects of flavonoids and mulch on biodiversity indices were related to sampling terms. Straw mulching increased

potentially antagonistic fungi in pea rhizosphere. The results of this study can be useful in understanding the effects of flavonoids and mulch on microbial activity and dynamics in pea rhizosphere which is very important in soil quality and crop production.

**Keywords** Antagonistic fungi · Functional diversity · *Pisum sativum* · Rhizosphere · Soil enzymes

## Introduction

Flavonoids are secondary metabolites that are released by leafs and plant roots into the surrounding soil through root exudation and as a result of root decomposition, turnover and injury (Singh and Prasad 2016). They play a very important role in rhizobia–legumes symbiosis (D'Haese and Holsters 2002). Specific flavonoids released by legume roots attract the rhizobial symbiont and activate expression of rhizobial genes, which are responsible for production of the molecule (Nod factors), that induce infection with rhizobia and nodule formation. Up to now, several studies tested the efficacy of external application of flavonoids on plant growth. Pre-incubation of *R. leguminosarum* with flavonoids prior to inoculation of pea seeds caused beneficial effects on the nodulation and pod numbers (Begum et al. 2001). In other study, pre-induction of *R. leguminosarum* by *trifolii* with clover exudates-contained flavonoids increased clover wet mass of shoots and nodule number (Maj et al. 2010).

Flavonoids play also a multifunctional role in plant–microbe communication and microbial growth and activity. Flavonoids have been shown to protect plant from diseases, influenced on N, P, S and micronutrients cycles (Cesco et al. 2012). It was indicated that flavonoids may play a role

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as early signals for both rhizobia and arbuscular mycorrhizal fungi AMF (Antunes et al. 2006). Concentrations as small as  $1 \mu\text{g g}^{-1}$  dry soil were sufficient to elicit changes in microbial community structure (Guo et al. 2011). A positive correlation between daidzein concentrations and soil fungi was noted. The genistein concentration showed a correlation with the total PLFA, fungi, bacteria, Gram (+) bacteria and aerobic bacteria in the soil microbial community (Guo et al. 2011). Under field conditions, microbial biomass carbon positively correlated both with the concentrations of genistein and daidzein in the rhizosphere (Wang et al. 2012).

Agricultural practices influence soil microorganisms quantity and community structure and soil microbial processes through changes in the plant residues entering the soil, nutrient input and soil biochemical and physical properties. Mulching with plant residues influenced a number of soil properties such as: organic matter content, nutrient availability, structure, porosity, moisture and temperature status. Thus, application of organic mulch may affect crop development and microbial activity. Abdullah (2014) observed beneficial effects of wheat residue incorporation on soil water content, soil organic matter, seed yield and seed oil content of canola compared with no residue treatment. The yields of seeds, protein, oil and water productivity of soybean seeds and biomass were improved by surface straw mulching in years with well rainfall distribution during growing season (Siczek et al. 2015b). However, in growing season with extended period without or scarce rainfalls, mulch reduced growth and yield of soybean. Enhanced content of microbial biomass and its activity was noted by Tu et al. (2006) under straw mulched in relation to non-mulched soil. The plots with addition of straw had higher values of enzymatic activity, microbial biomass and respiration, reaching similar values to soil under native vegetation (García-Orenes et al. 2010). However, straw mulch effect on enzymes activities was strongly dependent on mulching levels and some adverse effects at straw mulching higher than  $15,000 \text{ kg ha}^{-1}$  were observed (Zhang et al. 2015). Straw mulch effect on soil enzymatic activity was also dependent on soil compaction level (Siczek and Frac 2012). The study of Govaerts et al. (2007) indicated that soil microbial biomass C and N was significantly higher for residue retention compared to residue removal in a long-term field experiment. Similarly, the average well colour development (AWCD) obtained by the Biolog<sup>TM</sup> EcoPlate assay was significantly higher for residue retention compared to residue removal.

From the literature review, it appears that flavonoids and straw mulch are of great importance for soil microbe population and activity, and, as consequence, plant growth and yields. However, according to our knowledge, no studies on the effects of flavonoids on microbial functional

diversity in pea rhizosphere were performed. The effects of flavonoids may be modulated by straw mulch and so far these effects are not recognized. Therefore, the aim of this study was to determine the effects of flavonoids and straw mulch on the rhizosphere soil microbial parameters such as enzymes activities, microbial functional diversity and abundance during pea growing season. The experiment was performed in Lublin, Poland ( $51^{\circ}15'N$ ,  $22^{\circ}35'E$ ) in 2013.

## Materials and methods

### Description of the study site and treatments

The field study was conducted in Lublin, Poland ( $51^{\circ}15'N$ ,  $22^{\circ}35'E$ ). The soil was a Haplic Luvisol (according to the FAO–Unesco, World Reference Base 2006). The field was under long-term (30 years) conventional tillage. Main tillage operations included pre-plough, harrowing and mouldboard ploughing (20–25 cm depth). Characteristics of the 0–20-cm soil layer are presented in Table 1. The study factors were as follows: flavonoids (*F*) and straw mulch (*M*). Pea seeds (*Pisum sativum* L. cv. Tarchalska) were soaked for 30 min with flavonoids (*F*) or water (control, *C*) and planted. Three–four days after sowing, a straw mulch (chopped into 3–5-cm long pieces) was applied on the soil surface at a rate of  $0.5 \text{ kg m}^{-2}$ . Study treatments were as follows: *C* (control), *F* (flavonoids), *M* (straw mulch) and *FM* (flavonoids + straw mulch). The plots (2 m × 3 m) were organized in four replicates, following the randomized complete block design.

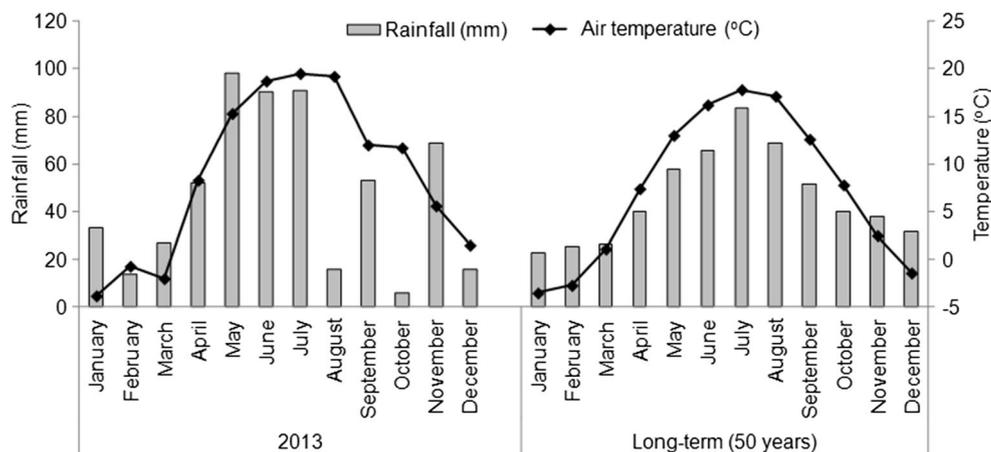
Rainfall and air temperature data were obtained from the meteorological station of the Institute of Agrophysics, Polish Academy of Sciences in Lublin, located 160 m from the experimental site. In reference to the long-term average (552 mm), yearly precipitations in 2013 were similar (565 mm) (Fig. 1). However, the amount of rainfall during the pea growing season (April–July) was higher in 2013 than the long-term average (by 25%). The mean air temperatures during the pea growing seasons in 2013 and long term were 8.8 and 7.4 °C, respectively.

**Table 1** Properties of the 0–20-cm soil layer

Clay $\text{g kg}^{-1}$	Silt	Sand	C org	Total $N^a$	$P^b$ $\text{mg kg}^{-1}$	K $\text{mg kg}^{-1}$	Mg	pH $H_2O$
70	290	640	14.1	0.75	90	153	23	5.9

<sup>a</sup> As indicated by the Kjeldahl method

<sup>b</sup> Plant available inorganic P

**Fig. 1** Mean monthly rainfalls and air temperatures

### Preparation of flavonoids

Flavonoids were extracted from sprouted pea seeds (Siczek et al. 2015a). Briefly, surface sterilized seeds were shaken in darkness and then flavonoids were extracted with ethyl acetate. After evaporation of ethyl acetate, the pellet with flavonoids was resolubilized in 95% ethanol and stored at 4 °C. The UV–VIS analysis indicated the presence of isoflavones (e.g., genistein and/or biochanin A) in the exudates. The flavonoids concentration used in this study amounted to 32.2 µM.

### Sampling of rhizosphere soil

Samples for microbial analyses were taken from the soil layer of 0–15 cm, three times during the vegetative period of pea: T1 five–six leaf stage, T2 flowering and T3 pod formation. Loosely adhering soil to the roots was shaken off, and then the roots were vigorously shaken. The soil dislodged from the roots was collected as rhizosphere soil. Soil was sieved through a 0.2-cm mesh sieve and used for measurements or stored at 4 °C.

### Biochemical and microbiological analysis

Dehydrogenases activity was determined by the Thalmann (1968) method, modified by Alef (1995), with 2,3,5 triphenyl-tetrazolium chloride (TTC) as a substrate. Protease activity was determined according to the Ladd and Butler (1972) method, modified by Alef and Nannipieri (1995), with soil incubation with a sodium caseinate solution. Tabatabai and Bremner (1969) method was applied for acid phosphomonoesterase activity measurement after soil incubation with p-nitrophenyl disodium phosphate. Enzyme analysis was done in three replicates per treatment.

Metabolic potential of soil bacteria communities was evaluated using Biolog EcoPlate™ (Biolog Inc., Hayward,

CA, USA) with a 31 carbon sources (Insam 1997). Each well of the Biolog EcoPlate™ was inoculated with 120 µL of rhizosphere soil inoculum and incubated at 27 °C. Absorbance readings were taken every 24 h for 72 h at 590 nm with a plate reader Biolog MicroStation™. On the basis of data obtained at 72 h, Richness (*S*), Shannon–Weaver (*H*), *E* (Oszust et al. 2014) and average well colour development (AWCD) indexes were calculated following Garland and Mills (1991).

The numbers of cultivable bacteria and fungi were determined with the plate method. A medium with soil extract and K<sub>2</sub>HPO<sub>4</sub>, *Pseudomonas* F Lab-agar, Trypticasein Soy Lab-agar and Martin (1950) media were used for total bacteria, *Pseudomonas*, *Bacillus* and fungi count, respectively. Microbe populations were assessed in three repetitions for each treatment. Results were based on oven-dry (105 °C) weight of soil.

The populations of predominant fungal species in pea rhizosphere were identified based on morphotypic identification of fungal isolates (Frac et al. 2014). Strains were identified at the genus level. Cultures were incubated at 26 °C for 14 days on different Czapek agar and potato dextrose agar (Biocorp) in three repetitions for each treatment. Macroscopic observation included the colony diameter and shape, conidia colours and level of sporulation. Microscopic observations of the mycelia and conidia were done by an optical microscope. Genus identification was performed using the systematic classification methods of Domsch et al. (1980) and Watanabe (2010).

### Statistical analysis

Statistical analyses were performed with Statistica 10.0 software (StatSoft Inc., Tulsa, OK, USA, 2011). Collected data have been subjected to three-way (enzymes activities, microbial populations, biodiversity indices from EcoPlate) analysis of variance (ANOVA) for the comparison of means, and significant differences were calculated



according to post hoc Tukey's HSD (honestly significant differences) test at  $P < 0.05$  significant level. Cluster analysis, including grouping of treatments and features, was performed on standardized data from the average absorbance values at 72 h (Biolog EcoPlate™). To present the similarity of the carbon utilization patterns of the substrates that were located on the Biolog EcoPlate™ between the treatments, a dendrogram was prepared with scaled bond distances on the axis (Ward's method) and boundary marked according to Sneath's criteria (33 and 66%). The data were standardized according to AWCD in each microplate to remove the inoculum density effects (Garland 1997). The principal component analysis (PCA) was performed for better understanding of the relationship between treatments and biochemical and biological indicators.

## Results and discussion

The present study was designed to determine the effects of flavonoids and straw mulch on soil enzymes activity and microbes population and activity in the pea rhizosphere. The rhizosphere, which is the zone of soil that is influenced by root secretions, is of central importance for plant nutrition, health and quality, stress tolerance and nutrient cycling.

### Soil enzymatic activity

The results revealed significant and positive effect of flavonoids and mulch ( $F$  and  $FM$  treatment) on dehydrogenases and protease activities (Table 2). Flavonoids significantly improved dehydrogenases activity at T2 (48%) and T3 (20%), whilst mulch—at T2 (by 78%), in relation to  $C$ . Comparing to control,  $FM$  treatment showed higher activities of dehydrogenases at T2 and T3, and protease at T2 (by 66, 60 and 63%, respectively). When annual averages taken into consideration, dehydrogenases and protease were significantly higher than control under all treatments (in the range of 30–55%), except protease under  $M$  treatment. Acid phosphatase did not significantly differ between treatments. The most pronounced improvement in relation to  $C$  was noticed under  $FM$  treatment for both dehydrogenases (55%) and protease (37%).

Thus, application of flavonoids on pea seed and soil mulching may have considerable importance in N cycling and supply of nutrients to the microbial and plant growth. Dehydrogenases that occur intracellular in all living microbial cell play a significant role in the biological oxidation of soil organic matter (OM) by transferring hydrogen from organic substrates to inorganic acceptors (Zhang et al. 2010). As regards protease, its activity is an

important with regard to N cycling in ecosystems, as proteolysis is considered to be a rate-limiting step during N mineralization in soils. It has been estimated that about 40% of the total soil N is proteinaceous (Schulten and Schnitzer 1998). These results support previous research into this area on beneficial effects of mulch on soil activity (Tu et al. 2006; García-Orenes et al. 2010). Under field conditions, mulched soybean showed greater values of dehydrogenases, protease, and acid and alkaline phosphatases than unmulched soil (Siczek and Frac 2012).

### Microbial functional diversity

The effects of  $F$  and  $M$  on biodiversity indices of soil bacteria was related to sampling terms (Table 3). AWCD were significantly lower under  $F$  at T1 and T3 (by 25 and 54%) as  $R$  (10, 25%), whilst  $E$  was significantly greater at T2 and T3 (4–6%) than in  $C$ . As regards both mulched treatments, significantly greater AWCD were noted at T1 and T3 (24–45%), and lower (14–16%) at T2.  $H$  and  $E$  increased ( $P < 0.05$ ) in mulched treatments comparing to  $C$  at T2. After flavonoids application mean values of AWCD and  $R$  were significantly lower than under  $C$  (25 and 11%, respectively), and  $E$  was greater by 4%. There were no significant differences between  $M$  and  $C$  treatments as regards mean values of biodiversity indices.

Figure 2 presents the bond distances between the treatments, where clustering was assessed by the carbon substrates utilization. Taking into consideration Sneath's criterion 33%, four groups (A–D) were distinguished. A similar response was noted under almost all mulched treatments, except  $FM$  at T3 (group B) in terms of carbon substrate utilization. Taking into consideration less restrictive Sneath's criterion 66%, 2 groups were found (1 and 2). All mulched treatments and objects from T2 sampling time were in group 1, and  $C$  and  $F$  treatments at T1 and T3 belonged to group 2.

### Microbial abundance

Growth of fungi was impeded under  $F$  in relation to  $C$  at T3 (34%) (Table 4). It could be seen that applications of mulch significantly hampered growth of fungi at T2 (by 71%) and greatly enhanced growth of *Bacillus* at T1 and T2, and *Pseudomonas* at T3 (172, 31 and 84%, respectively), when compared with control. Under  $FM$ , stimulation of fungi and *Bacillus* abundance at T2 in relation to both  $F$  and  $M$  treatments was noted. At all terms, both  $F$  and  $M$  did not affect total number of bacteria. Annual averages were significantly higher than control under  $M$  (*Bacillus* and *Pseudomonas*) and under  $FM$  (*Bacillus*) and lower than  $C$  under  $F$  and  $M$  (fungi). Mulch and time affected significantly all analysed bacterial groups (Table 5).

**Table 2** Enzymatic activities

Time	Treatment	Dehydrogenases ( $\text{cm}^3 \text{H}_2 \text{kg}^{-1} \text{d}^{-1}$ )	Protease (mg tyrosine $\text{kg}^{-1} \text{h}^{-1}$ )	Acid phosphomonoesterase (mg PNP $\text{kg}^{-1} \text{h}^{-1}$ )
T1	<i>C</i>	3.52 f	2.51 e	24.22 d
	<i>F</i>	4.44 ef	3.62 cde	27.88 cd
	<i>M</i>	4.16 ef	3.68 cde	24.46 d
	<i>FM</i>	4.57 ef	3.69 cde	26.58 cd
T2	<i>C</i>	5.30 e	3.08 de	36.91 a
	<i>F</i>	7.82 d	4.63 bcd	34.20 ab
	<i>M</i>	9.44 dc	3.60 cde	36.94 a
	<i>FM</i>	8.80 bcd	5.02 abc	34.65 a
T3	<i>C</i>	8.23 cd	5.41 ab	32.57 ab
	<i>F</i>	9.91 b	6.16 ab	30.22 bc
	<i>M</i>	9.29 bc	5.48 ab	36.69 a
	<i>FM</i>	13.13 a	6.39 a	33.85 ab
Mean	<i>C</i>	5.68 c	3.67 c	31.2 a
	<i>F</i>	7.39 b	4.80 ab	30.8 a
	<i>M</i>	7.63 b	4.26 bc	32.7 a
	<i>FM</i>	8.83 a	5.03 a	31.7 a

Different letters within the same parameters indicate statistical differences ( $P < 0.05$ ). T1, T2, T3, 5–6 leaf, flowering and pod formation stages of pea development, respectively

*C* control, *F* flavonoids, *M* straw mulch

**Table 3** Biodiversity indices for bacterial community

Time	Treatment	AWCD	<i>S</i>	<i>H</i>	<i>E</i>
T1	<i>C</i>	0.723 f	28.0 b	3.31 abc	0.99 b
	<i>F</i>	0.545 g	25.3 c	3.27 bc	1.01 b
	<i>M</i>	0.896 cde	29.3 ab	3.35 abc	0.99 b
	<i>FM</i>	0.924 bcd	29.3 ab	3.36 ab	0.99 b
T2	<i>C</i>	1.00 ab	29.7 a	3.25 c	0.96 c
	<i>F</i>	0.96 abc	30.3 a	3.40 a	1.00 b
	<i>M</i>	0.86 de	30.0 a	3.39 a	1.00 b
	<i>FM</i>	0.84 de	29.0 ab	3.36 ab	1.00 b
T3	<i>C</i>	0.71 f	29.7 a	3.36 ab	0.99 b
	<i>F</i>	0.33 h	22.3 d	3.25 c	1.05 a
	<i>M</i>	0.81 ef	29.0 ab	3.30 abc	0.98 bc
	<i>FM</i>	1.03 a	30.0 a	3.38 a	0.99 b
Mean	<i>C</i>	0.814 b	29.1 a	3.31 a	0.98 b
	<i>F</i>	0.611 c	26.0 b	3.31 a	1.02 a
	<i>M</i>	0.854 b	29.4 a	3.35 a	0.99 b
	<i>FM</i>	0.931 a	29.4 a	3.37 a	0.99 b

Different letters within the same parameters indicate statistical differences ( $P < 0.05$ ). T1, T2, T3 5–6 leaf, flowering and pod formation stages of pea development, respectively

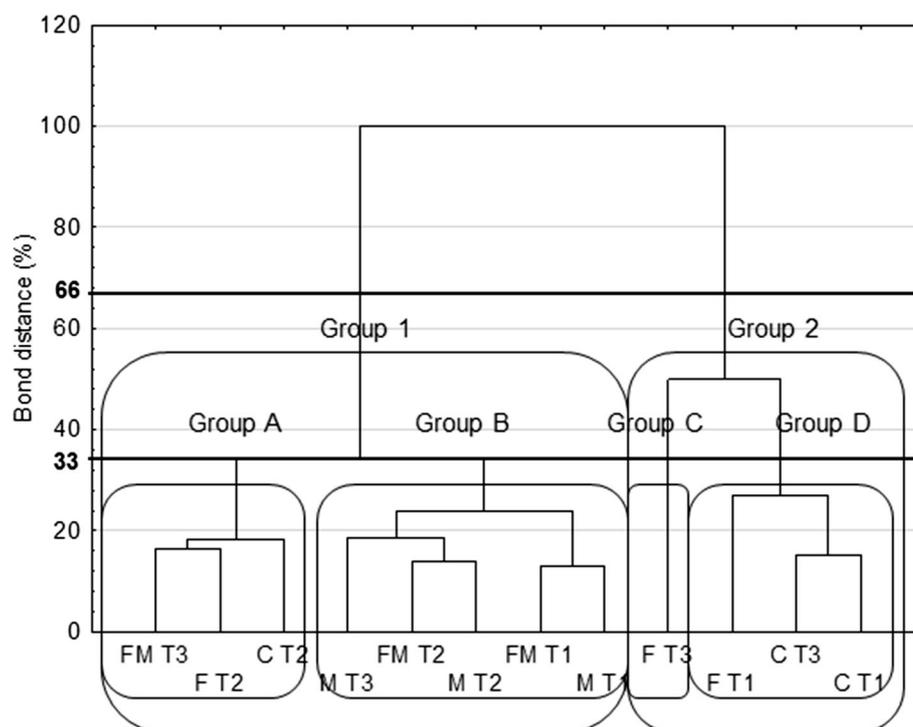
*C* control, *F* flavonoids, *M* straw mulch, AWCD average well colour development, *S* Richness, *H* Shannon–Weaver, *E* Evenness

The current study found that application of straw mulch enhanced the growth of *Bacillus* and *Pseudomonas* spp. that are known as plant growth-promoting rhizobacteria

(PGPR). These beneficial rhizosphere microorganisms can inhibit the growth or activity of soil-borne pathogenic fungi and bacteria through adverse affect on the population density, dynamics and metabolic activities (Raaijmakers et al. 2009). Different *Pseudomonas* and *Bacillus* strains showed antibacterial, antifungal or antagonistic activity against plant pathogens (Sturz and Christie 2003; Ahmadzadeh and Tehrani 2009; Sang and Kim 2012). Some species of this bacteria positively affected legume growth, yield and symbiotic activity (Ahmadzadeh and Tehrani 2009; Zahir et al. 2011). Thus, mulch application may limit the activity of plant pathogens, modulate microbial activities and might lead to a improvement of pea growth and yield. Similar results were obtained by Tiquia et al. (2002) who observed that the population of culturable rhizosphere *Pseudomonas* was significantly higher in soil from plots mulched with compost than in the bare soil.

In response to growth stimulation by quercetin or other flavonoids released by plants, many bacteria and fungi may multiply (Hartwig and Phillips 1991; Guo et al. 2011; Wang et al. 2012). In contrast to those findings, however, no evidence of positive effect of flavonoids on bacterial population in our study was indicated. However, a trend in increasing all analysed bacterial population by *F* at T1 and decreasing at T2 was noted. *F* significantly reduced fungi number at T3. The effect of flavonoids on microbial community structure in

**Fig. 2** Dendrogram of the bond distances between the carbon utilizations patterns of the substrates located on the Biolog EcoPlates™. Grouping was conducted according to the Sneath's criteria 33 and 66%. *C* control, *F* flavonoids, *M* straw mulch, T1, T2, T3 5–6 leaf, flowering and pod formation stages of pea development, respectively



**Table 4** Number of microorganisms

Time	Treatment	Fungi (CFU 10 <sup>3</sup> g <sup>-1</sup> )	Total bacteria (CFU 10 <sup>7</sup> g <sup>-1</sup> )	<i>Bacillus</i> (CFU 10 <sup>7</sup> g <sup>-1</sup> )	<i>Pseudomonas</i> (CFU 10 <sup>7</sup> g <sup>-1</sup> )
T1	<i>C</i>	19.9 e	72.3 a	60.3 h	86.2 bcd
	<i>F</i>	30.0 e	175.9 a	82.8 gh	110.2 bc
	<i>M</i>	38.8 e	69.5 a	164.0 def	137.1 ab
	<i>FM</i>	47.6 e	232.8 a	177.2 de	51.1 d
T2	<i>C</i>	530.7 bc	262.5 a	264.8 c	85.0 bcd
	<i>F</i>	397.9 cd	169.8 a	213.3 cd	67.9 cd
	<i>M</i>	153.5 de	307.1 a	347.0 b	72.7 cd
	<i>FM</i>	791.3 ab	262.1 a	443.5 a	79.6 cd
T3	<i>C</i>	971.8 a	75.9 a	113.9 efgh	89.6 bcd
	<i>F</i>	641.2 bc	138.4 a	84.5 fgh	102.0 bcd
	<i>M</i>	840.3 ab	287.3 a	122.8 efgh	164.5 a
	<i>FM</i>	1094.9 a	96.4 a	143.9 defg	169.4 a
Mean	<i>C</i>	507.5 a	136.9 a	146.3 c	86.9 b
	<i>F</i>	356.4 b	161.4 a	126.9 c	93.4 b
	<i>M</i>	344.2 b	221.3 a	211.3 b	124.8 a
	<i>FM</i>	644.6 a	197.1 a	254.9 a	100.1 b

Different letters within the same parameters indicate statistical differences ( $P < 0.05$ ). T1, T2, T3 5–6 leaf, flowering and pod formation stages of pea development, respectively

*C* control, *F* flavonoids, *M* straw mulch

the soil is connected with their role as a carbon source for some species as well as they can inhibit the growth of others due to their phytoalexin properties (Walker et al. 2003; Hassan and Mathesius 2012). In this study,

cultivable methods for counting of bacteria and fungi were used. Further research should be undertaken for assessment structure and diversity of unculturable microbes with molecular techniques.

**Table 5** *p* values of microbial parameters

Dependent variable	<i>F</i>	<i>M</i>	<i>T</i>	<i>F</i> × <i>M</i>
Dehydrogenases (cm <sup>3</sup> H <sub>2</sub> kg <sup>-1</sup> d <sup>-1</sup> )	<0.001	<0.001	<0.001	0.135
Protease (mg tyrosine kg <sup>-1</sup> h <sup>-1</sup> )	<0.001	0.033	<0.001	0.330
Acid phosphomonoesterase (mg PNP kg <sup>-1</sup> h <sup>-1</sup> )	0.153	0.025	<0.001	0.594
Biodiversity indices				
AWCD	0.002	<0.001	<0.001	<0.001
<i>R</i>	<0.001	<0.001	<0.001	<0.001
<i>H</i>	0.690	0.026	0.566	0.622
<i>E</i>	0.003	0.246	0.113	0.021
Fungi (CFU 10 <sup>3</sup> g <sup>-1</sup> )	0.048	0.095	<0.001	<0.001
Total bacteria (CFU 10 <sup>7</sup> g <sup>-1</sup> )	0.995	0.044	0.006	0.399
<i>Bacillus</i> (CFU 10 <sup>7</sup> g <sup>-1</sup> )	0.200	<0.001	<0.001	0.002
<i>Pseudomonas</i> (CFU 10 <sup>7</sup> g <sup>-1</sup> )	0.137	0.001	<0.001	0.015

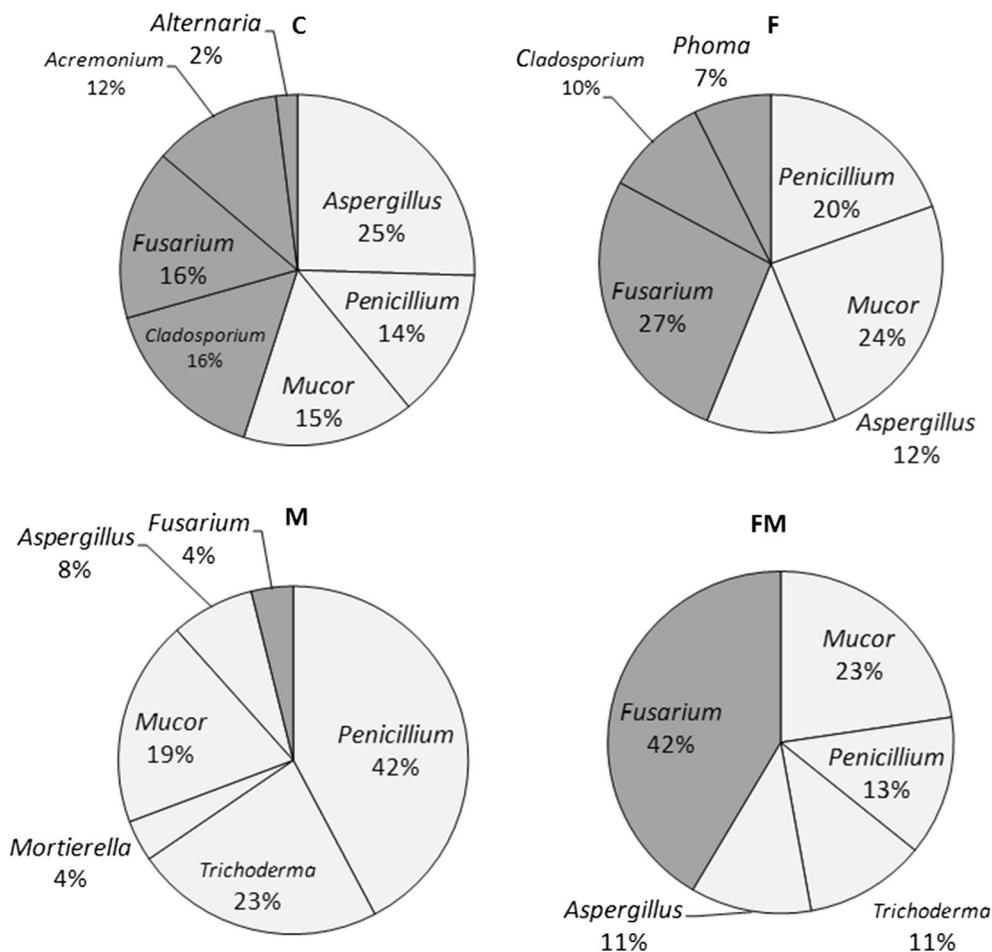
*F* flavonoids, *M* straw mulch, *T* sampling time, AWCD average well colour development, *S* Richness, *H* Shannon–Weaver, *E* Evenness

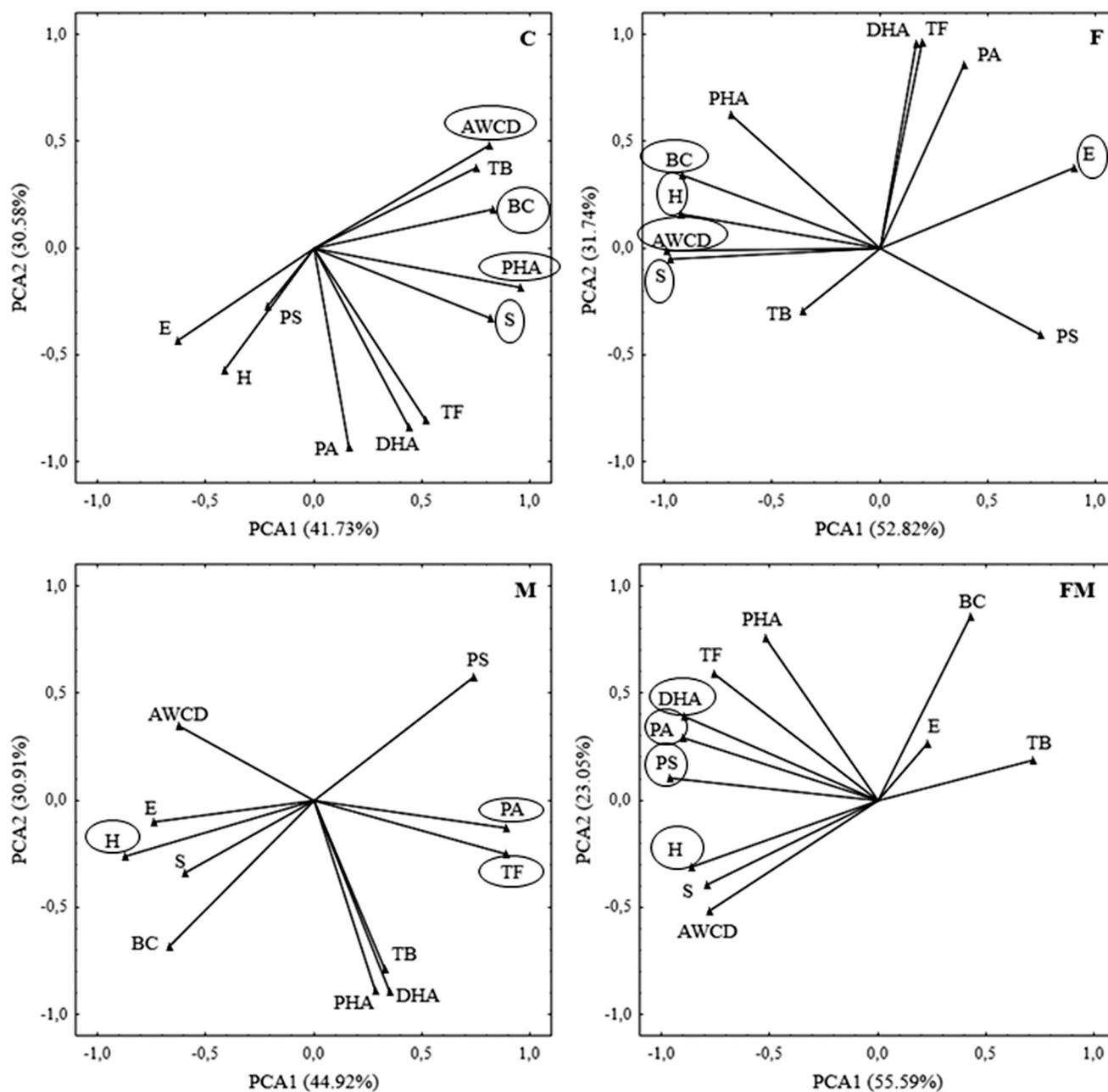
**Pea rhizosphere fungi**

As shown in Fig. 3, flavonoids and mulch caused changes in dominant fungal communities. From pea rhizosphere potentially phytopathogenic (*Fusarium*, *Cladosporium*,

*Acremonium*, *Alternaria* and *Phoma*) and potentially antagonistic (*Penicillium*, *Aspergillus*, *Mucor*, *Trichoderma* and *Mortierella*) genus were isolated. From 4 (*M*) to 46% (*C*) phytopathogenic and from 54 (*C*) to 96% (*M*) antagonistic isolates were noted. The greatest

**Fig. 3** Average (from T1, T2 and T3) frequency (%) of predominant fungal genus in pea rhizosphere. Bright colour indicates potentially antagonistic fungi; grey colour indicates potentially phytopathogenic fungi. C control, F flavonoids, M straw mulch





**Fig. 4** PCA based on soil microbial variations for particular treatments. *C* control, *F* flavonoids, *M* straw mulch, *TB* total bacteria, *BC* *Bacillus*, *PS* *Pseudomonas*, *TF* total fungi, *PHA*

phosphomonoesterase activity, *DHA* dehydrogenases activity, *PA* protease activity, *AWCD* average well colour development, *S* Richness, *H* Shannon–Weaver, *E* Evenness

differences in relation to control were observed under *M*. Mulch beneficially affected predominant fungal genera by reducing the incidence and diversity of phytopathogenic fungi, and increasing the incidence and diversity of antagonistic fungi.

Application of mulch results in appearance of *Trichoderma* spp. among major fungi isolates in the rhizosphere. It was shown that *Trichoderma* showed biocontrol effects on soil-borne plant pathogens through secretion of antimicrobial secondary metabolites and chitinases and

cellulases (Raaijmakers et al. 2009). It may be expected that the high population of the antagonistic *Bacillus* spp., *Pseudomonas* spp. and *Trichoderma* spp. in the mulch-treated soils led to a substantial reduction of the population of phytopathogens.

The beneficial effect of mulch on the bacterial population and enzymes activities could be related with the influence on soil physical and chemical characteristics (Jin et al. 2009). Under the same climatic conditions, straw mulch lowered the daily temperature and moisture

fluctuation (Siczek et al. 2015b). It was shown that soil temperature significantly affected the count of soil microorganisms and enzymes activities (Borowik and Wyszowska 2016). Another possible explanation for changes the microbial activity during pea vegetation is decomposition of carbon compounds derived from the residues and leached through the soil profile because of rain (Coppens et al. 2006; Pascault et al. 2010). Seasonal variation of AWCD from EcoPlates was observed under mulched soils: at T1 and T3 mulch increased ( $P < 0.05$ ), and at T2 decreased its values when comparing with the control.

It is interesting to note that when flavonoids and mulch were used simultaneously, the effects on some parameters, like fungi and *Bacillus* numbers, AWCD, dehydrogenases and protease activities increased significantly in relation to the treatments with those factors used separately. However, some other parameters like *E* and *Pseudomonas* number were lower.

The principal component ordination based on soil variables for particular treatments was done to notice differences among flavonoids and mulch application within the microbiological indicators. The results for soil microbial variables showed overall differences among treatments (Fig. 4). The trend of microbial variables in control soil and treatment with flavonoids was similar showing the highest influence of applied factors on average well colour development (AWCD), *Bacillus* (BC) and richness (*S*). The values of dehydrogenases (DHA), protease (PA) and *Pseudomonas* (PS) parameters had a great contribution for discrimination of the treatment with flavonoids and straw mulch as it was not significantly related across the other treatments investigated. However, only Shannon index (*H*) was considered as common indicator among all tested treatments as it was not related to the control soil. Moreover, the distinction of *C*, *F*, *M* and *FM* seems to be driven by contrasts between bacterial variables (PS, BC, AWCD) along the variation axis. The PCA results showed that soil microbial variables significantly related to ordination axes contribute the importance of these variables.

## Conclusion

In summary, the results from this study proved that mulching increases potentially antagonistic fungi in pea rhizosphere. The results of this study help understand the effects of flavonoids and mulch on microbial activity and dynamics in pea rhizosphere, which can be useful for the development of suitable way to manage microbial processes and, as a consequence, soil quality and crop production.

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## References

- Abdullah AS (2014) Minimum tillage and residue management increase soil water content, soil organic matter and canola seed yield and seed oil content in the semi arid areas of Northern Iraq. *Soil Tillage Res* 144:150–155
- Ahmadzadeh M, Tehrani AS (2009) Evaluation of fluorescent pseudomonads for plant growth promotion, antifungal activity against *Rhizoctonia solani* on common bean, and biocontrol potential. *Biol Control* 48(2):101–107
- Alef K (1995) Dehydrogenase activity. In: Alef K, Nannipieri P (eds) *Methods in applied soil microbiology and biochemistry*. Academic Press, London
- Alef K, Nannipieri P (1995) Protease activity. In: Alef K, Nannipieri P (eds) *Methods in applied soil microbiology and biochemistry*. Academic Press, London
- Antunes PM, Rajcan I, Goss MJ (2006) Specific flavonoids as interconnecting signals in the tripartite symbiosis formed by arbuscular mycorrhizal fungi, *Bradyrhizobium japonicum* (Kirchner) Jordan and soybean (*Glycine max* (L.) Merr.). *Soil Biol Biochem* 38(3):533–543
- Begum AA, Leibovitch S, Migner P, Zhang F (2001) Specific flavonoids induced nod gene expression and pre-activated nod genes of *Rhizobium leguminosarum* increased pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.) nodulation in controlled growth chamber environments. *J Exp Bot* 52(360):1537–1543
- Borowik A, Wyszowska J (2016) Impact of temperature on the biological properties of soil. *Int Agrophys* 30(1):1–8
- Cesco S, Mimmo T, Tonon G, Tomasi N, Pinton R, Terzano R, Neumann G, Weisskopf L, Renella G, Landi L, Nannipieri P (2012) Plant-borne flavonoids released into the rhizosphere: impact on soil bio-activities related to plant nutrition. A review. *Biol Fertil Soils* 48(2):123–149
- Coppens F, Garnier P, De Gryze S, Merckx R, Recous S (2006) Soil moisture, carbon and nitrogen dynamics following incorporation and surface application of labelled crop residues in soil columns. *Eur J Soil Sci* 57(6):894–905
- D'Haese W, Holsters M (2002) Nod factor structures, responses, and perception during initiation of nodule development. *Glycobiology* 12(6):79–105
- Domsch KH, Gams W, Anderson TH (1980) *Compendium of soil fungi*. Academic Press, Waltham
- Fraç M, Oszust K, Lipiec J, Jezierska-Tys S, Oluchi Nwaichi E (2014) Soil microbial functional and fungal diversity as influenced by municipal sewage sludge accumulation. *Int J Environ Res Public Health* 11(9):8891–8908
- García-Orenes F, Guerrero C, Roldán A, Mataix-Solera J, Cerdá A, Campoy M, Zornoza R, Bárcenas G, Caravaca F (2010) Soil microbial biomass and activity under different agricultural management systems in a semiarid Mediterranean agroecosystem. *Soil Tillage Res* 109(2):110–115



- Garland JL (1997) Analysis and interpretation of community-level physiological profiles in microbial ecology. *FEMS Microbiol Ecol* 24(4):289–300
- 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* 57(8):2351–2359
- Govaerts B, Mezzalama M, Unno Y, Sayre KD, Luna-Guido M, Vanherck K, Dendooven L, Deckers J (2007) Influence of tillage, residue management, and crop rotation on soil microbial biomass and catabolic diversity. *Appl Soil Ecol* 37(1–2):18–30
- Guo ZY, Kong CH, Wang JG, Wang YF (2011) Rhizosphere isoflavones (daidzein and genistein) levels and their relation to the microbial community structure of mono-cropped soybean soil in field and controlled conditions. *Soil Biol Biochem* 43(11):2257–2264
- Hartwig UA, Phillips DA (1991) Release and modification of nod-gene-inducing flavonoids from alfalfa seeds. *Plant Physiol* 95(3):804–807
- Hassan S, Mathesius U (2012) The role of flavonoids in root-rhizosphere signalling: opportunities and challenges for improving plant–microbe interactions. *J Exp Bot* 63(9):3429–3444
- Insam H (1997) A new set of substrates proposed for community characterization in environmental samples. In: Insam H, Ranggner A (eds) *Microbial communities. Functional versus structural approach*. Springer, Berlin, pp 259–260
- Jin K, Sleutel S, Buchan D, De Neve S, Cai DX, Gabriels D, Jin JY (2009) Changes of soil enzyme activities under different tillage practices in the Chinese Loess Plateau. *Soil Tillage Res* 104(1):115–120
- Ladd JN, Butler JHA (1972) Short-term assay of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. *Soil Biol Biochem* 4(1):19–30
- Maj D, Wielbo J, Marek-Kozaczuk M, Skorupska A (2010) Response to flavonoids as a factor influencing competitiveness and symbiotic activity of *Rhizobium leguminosarum*. *Microbiol Res* 165(1):50–60
- Martin JP (1950) Use of acid rose bengal and streptomycin in the plate method for estimating soil fungi. *Soil Sci* 69(3):215–232
- Oszust K, Frac M, Gryta A, Bilińska N (2014) The Influence of ecological and conventional plant production systems on soil microbial quality under hops (*Humulus lupulus*). *Int J Mol Sci* 15(6):9907–9923
- Pascault N, Nicolardot B, Bastian F, Thiébeau P, Ranjard L, Maron PA (2010) In situ dynamics and spatial heterogeneity of soil bacterial communities under different crop residue management. *Microb Ecol* 60(2):291–303
- Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, Moënne-Loccoz Y (2009) The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant Soil* 321(1):341–361
- Sang MK, Kim KD (2012) Plant growth-promoting rhizobacteria suppressive to *Phytophthora blight* affect microbial activities and communities in the rhizosphere of pepper (*Capsicum annuum* L.) in the field. *Appl Soil Ecol* 62:88–97
- Schulten HR, Schnitzer M (1998) The chemistry of soil organic nitrogen: a review. *Biol Fertil Soils* 26(1):1–15
- Siczek A, Frac M (2012) Soil microbial activity as influenced by compaction and straw mulching. *Int Agrophys* 26(1):65–69
- Siczek A, Frac M, Nawrocka A, Wielbo J, Kidaj D (2015a) The response of rhizosphere microbial properties to flavonoids and Nod factors. *Acta Agr Scand B-S P* 65(2):125–131
- Siczek A, Horn R, Lipiec J, Usowicz B, Lukowski M (2015b) Effects of soil deformation and surface mulching on soil physical properties and soybean response related to weather conditions. *Soil Tillage Res* 153:175–184
- Singh A, Prasad SM (2016) Nanotechnology and its role in agroecosystem: a strategic perspective. *Int J Environ Sci Technol*. doi:10.1007/s13762-016-1062-8
- Sturz AV, Christie BR (2003) Beneficial microbial allelopathies in the root zone: the management of soil quality and plant disease with rhizobacteria. *Soil Tillage Res* 72(2):107–123
- Tabatabai MA, Bremner JM (1969) Use of *p*-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol Biochem* 1(4):301–307
- Thalman A (1968) Zur Methodik der Bestimmung der Dehydrogenase—Aktivität im Boden mittels Triphenyltetrazoliumchlorid (TTC) [Methods of dehydrogenase activity determination with triphenyltetrazoliumchlorid (TTC)]. *Landwirtsch Forsch* 21:249–258 (in German)
- Tiquia SM, Lloyd J, Herms DA, Hoitink HAJ, Michel FC Jr (2002) Effects of mulching and fertilization on soil nutrients, microbial activity and rhizosphere bacterial community structure determined by analysis of TRFLPs of PCR—amplified 16S rRNA genes. *Appl Soil Ecol* 21(1):31–48
- Tu C, Ristaino JB, Hu S (2006) Soil microbial biomass and activity in organic tomato farming systems: effects of organic inputs and straw mulching. *Soil Biol Biochem* 38(2):247–255
- Walker TS, Bais HP, Grotewold E, Vivanco JM (2003) Root exudation and rhizosphere biology. *Plant Physiol* 132(1):44–51
- Wang J, Li X, Zhang J, Yao T, Wei D, Wang Y, Wang J (2012) Effect of root exudates on beneficial microorganisms—evidence from a continuous soybean monoculture. *Plant Ecol* 213(12):1883–1892
- Watanabe T (2010) Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species, 3rd edn. CRC Press, Boca Raton
- Zahir ZA, Zafar-ul-Hye M, Sajjad S, Naveed M (2011) Comparative effectiveness of *Pseudomonas* and *Serratia* sp. containing ACC-deaminase for coinoculation with *Rhizobium leguminosarum* to improve growth, nodulation, and yield of lentil. *Biol Fertil Soils* 47(4):457–465
- Zhang N, He XD, Gao YB, Li YH, Wang HT, Ma D, Zhang R, Yang S (2010) Pedogenic carbonate and soil dehydrogenase activity in response to soil organic matter in *Artemisia ordosica* community. *Pedosphere* 20(2):229–235
- Zhang X, Qian Y, Cao C (2015) Effects of straw mulching on maize photosynthetic characteristics and rhizosphere soil micro-ecological environment. *Chil J Agr Res* 75(4):481–487

