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Experimental evidence of soil bacteria abundance as the primary driver of rhizosphere priming effect

Evidencia experimental de la abundancia de bacterias del suelo como el principal iniciador del efecto de preparación de la rizosfera

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Abstract. Soil microbial communities are thought to be responsible for the rhizosphere priming effect (RPE). However, because soil microbial communities are comprised of diverse components, very little is known about which component plays the critical role. In this study, soybean and cottonwood were grown at two latitudinal locations with different temperature and light conditions in-situ. We quantified RPE using a natural $\delta^{13}C$ method, and measured the abundance, richness and composition of bacteria and fungi communities with DNA-based molecular methods. Among all potential variables, including the three aforementioned indexes of bacteria and fungi communities and soil physicochemical and plant indexes, bacterial abundance was found to explain a large proportion of the variation in RPE. Our study identified the biological mechanism underlying this important ecological process.

Keywords: Decomposition; Microbial diversity; Rhizosphere priming effect; Soil organic matter, Climate change.

Resumen. Se piensa que las comunidades microbianas del suelo son responsables del efecto de preparación de la rizósfera (RPE). Sin embargo, desde que las comunidades microbiales están compuestas de diversos componentes, se conoce muy poco acerca de cuál es el componente que tiene el rol principal en dicho efecto. En este estudio, se hicieron crecer soja y algodón en dos lugares a diferentes latitudes con diferentes condiciones de luz y temperatura in situ. Se cuantificó RPE usando un método natural de δC^{13} y se midió la abundancia, riqueza y composición de las comunidades de hongos y bacterias con métodos moleculares basados en el ADN. Entre todas las variables potenciales, incluyendo los tres índices de comunidades de hongos y bacterias anteriormente mencionados, e índices vegetales y físico-químicos del suelo, se mostró que la abundancia de bacterias explicó una gran proporción de la variación en RPE. Nuestro estudio identificó el mecanismo biológico que subyace este importante proceso ecológico.

Palabras clave: Descomposición; Diversidad microbial; Efecto de preparación de la rizósfera; Materia orgánica del suelo; Cambio climático.

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The presence of living plant roots can change various soil physicochemical indexes and microbial community activities, and these changes can further affect the decomposition rates of soil organic matter (SOM) (anywhere from a 70% reduction to a 380% enhancement in previous studies), which is known as the rhizosphere priming effect (RPE) (Kuzyakov, 2002; Zhu & Cheng 2011; Cheng et al., 2014). However, the detailed microbial groups responsible for RPE still remain underexplored, which should be primarily due to the high complexity of soil microbial communities and the prior limitation of technologies capable of quantifying microbial diversity as well as the RPE value (Kent & Triplett, 2002). In particular, the two dominant microbial groups, bacteria and fungi, have different community abundance, diversity and composition, but we do not know which group and community attribute are primarily driving RPE.

We conducted a study to investigate and quantify the potential microbial drivers of RPE. Briefly, two C_3 plant species, the N-fixing soybean and the non-N-fixing cottonwood, were grown in a "C_4-labelled soil", which was taken from a field under continuous maize cropping for 23 years (see details in the Supporting Method). Plants were grown in pots at two locations with 9° latitudinal difference, and thus with variable conditions of light and temperature; these pots were placed under light transmitting shelters to prevent the influence of precipitation and maintain constant soil moisture content. Control soil pots (without plants) were also maintained at both locations. At the 61st day (the flowering stage for soybean), soil respiration was measured using a closed circulation CO_2 trapping system. The $\delta^{13}C$ of root samples and that in the NaOH solutions were analyzed with cavity ring-sown spectroscopy (Picarro G2131-I Analyzer, Picarro Inc., USA). The rhizosfere priming effect was calculated with a two-source isotopic mixing linear model (Su et al. 2017). The abundances of bacterial 16S rRNA gene and fungi ITS sequences were quantified with real-time PCR. We adopted 454 pyrosequencing to sequence 16S rRNA gene and ITS sequence targets (PCR-amplification) for evaluating the taxonomic richness and composition of bacteria and fungi communities (Supporting Method).

Soybean caused positive RPEs at both locations of Heihe (high latitude: 50° 14' 38" N) and Shenyang (low latitude: 41° 47' 31" N) (Fig. 1), meaning that it stimulated the degradation of SOM. Cottonwood caused positive RPEs at Heihe but negative RPEs at Shenyang. During the experiment, there were shorter days (and thus, a smaller photosynthetic period) at the low latitude location of Shenyang relative to Heihe. This resulted in that the cottonwood biomass at Shenyang was only about 68.9% of that at Heihe. Thus, it is possible that soil microorganisms did not obtain enough carbon/energy resources from cottonwood at Shenyang, which could explain the negative RPEs. In contrast, the N-fixing soybean should have a higher photosynthetic efficiency than cottonwood (Kuzyakov & Domanski, 2000; Warembourg et al., 2003), so its RPEs were positive at both locations. Overall, plant species and experimental location caused a significant interacting effect (P<0.001, Fig. 1).

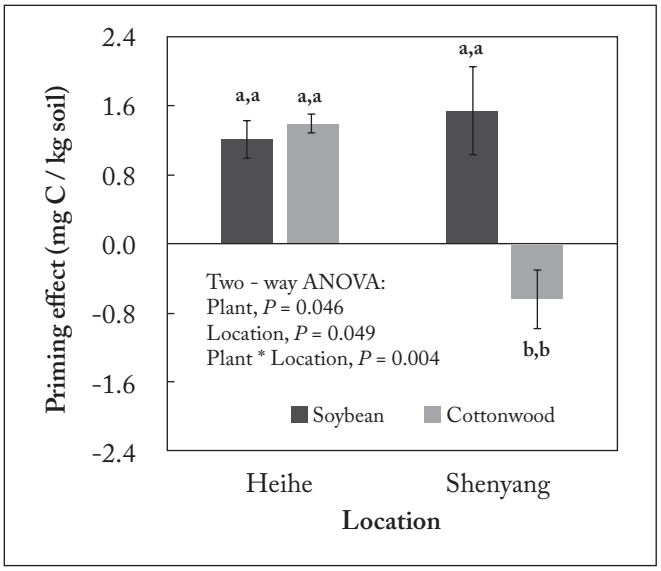

Fig. 1. Effect of different plant species and experimental location on the rhizosphere priming effect. Error bars indicate one standard error. The first letter before the comma above the histograms compares species within each location. The second letter after the comma above the histogram compares locations within each species.

Fig. 1. Efecto de diferentes especies vegetales y sitios experimentales en el efecto de preparación de la rizósfera. Las barras de error indican un error estándar. La primera letra antes de la coma compara especies dentro de cada sitio. La segunda letra después de la coma compara sitios dentro de cada especie.

The two factors of plant species and experimental location had significant effects on both the abundance and richness of soil bacteria communities (Fig. 2 a,b), and also influenced bacteria community composition - e.g., the communities with plants (treatments) and without plants (control) formed two distinct clusters in the NMDS plot (Fig. 2 c). Plant species also had a significant effect on the abundance of fungi communities, but had little effect on their richness and composition (Fig. 2 d-f). Taken together, bacteria seems to be more sensitive to these factors than fungi, consistent with many other studies (Di Lonardo et al., 2017).

All these variables of abundance, richness and composition of bacteria and fungi communities may be the biological driver of RPE. Since RPE reflects the differences in SOM degradation rate between pots with plants (treatments) and without plants (control), we first calculated the changes in the aforementioned six microbial indices between treatments and control, and then analyzed the relationships between RPE and these changes (Fig. 3). RPE was positively correlated with only the changes in bacteria abundance (P<0.05) (Fig. 3). Although

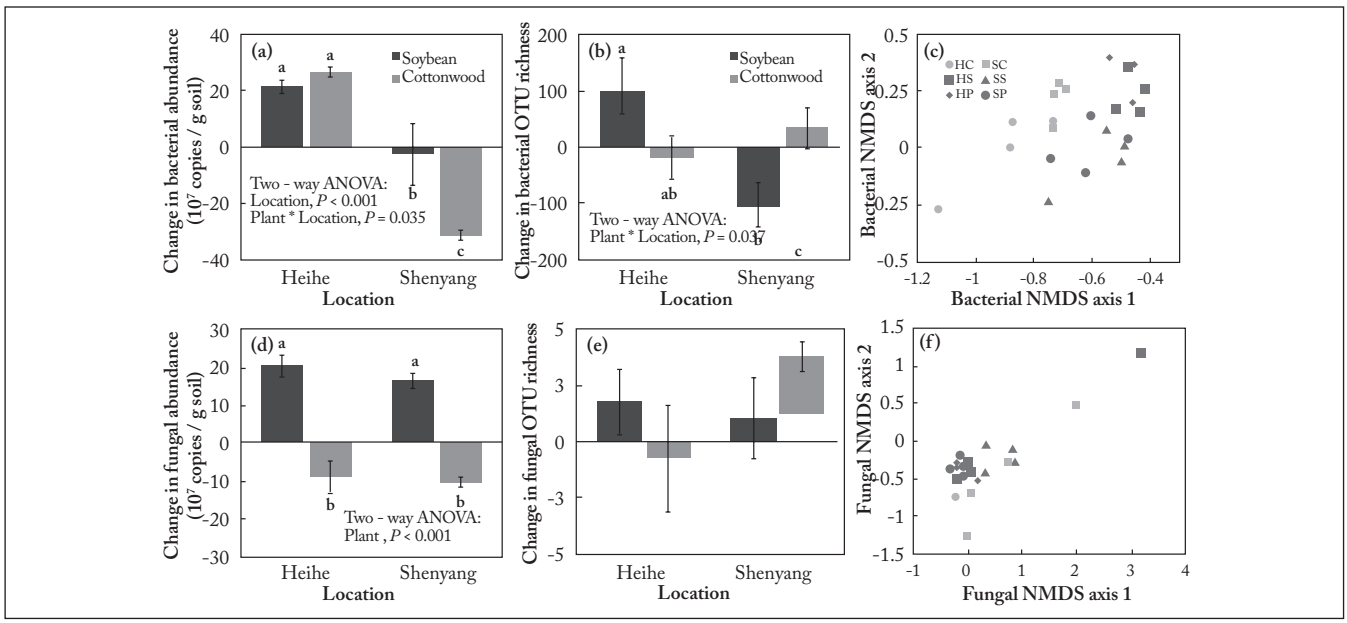

Fig. 2. Effect of different plant species and experimental locations on the abundance, richness and composition of soil bacteria and fungi communities. Error bars indicate one standard error. For clarity, only the significant results (P<0.05) are shown in the figures. HC, HS and HP represent control, soybean and cottonwood in Heihe city, respectively; and SC, SS and SP represent control, soybean and cottonwood in Shenyang city, respectively.

Fig. 2. Efecto de diferentes especies vegetales y sitios experimentales en la abundancia, riqueza y composición de las comunidades de hongos y bacterias de suelo. Las barras de error indicant un error estándar. Por razones de claridad, solo se muestran en las figuras los resultados significativos (P<0,05). HC, HS y HP representan el control, soja y algodón en la ciudad Heihe, respectivamente; y SC, SS y SP representan el control, soja y algodón en la ciudad de Shenyang, respectivamente.

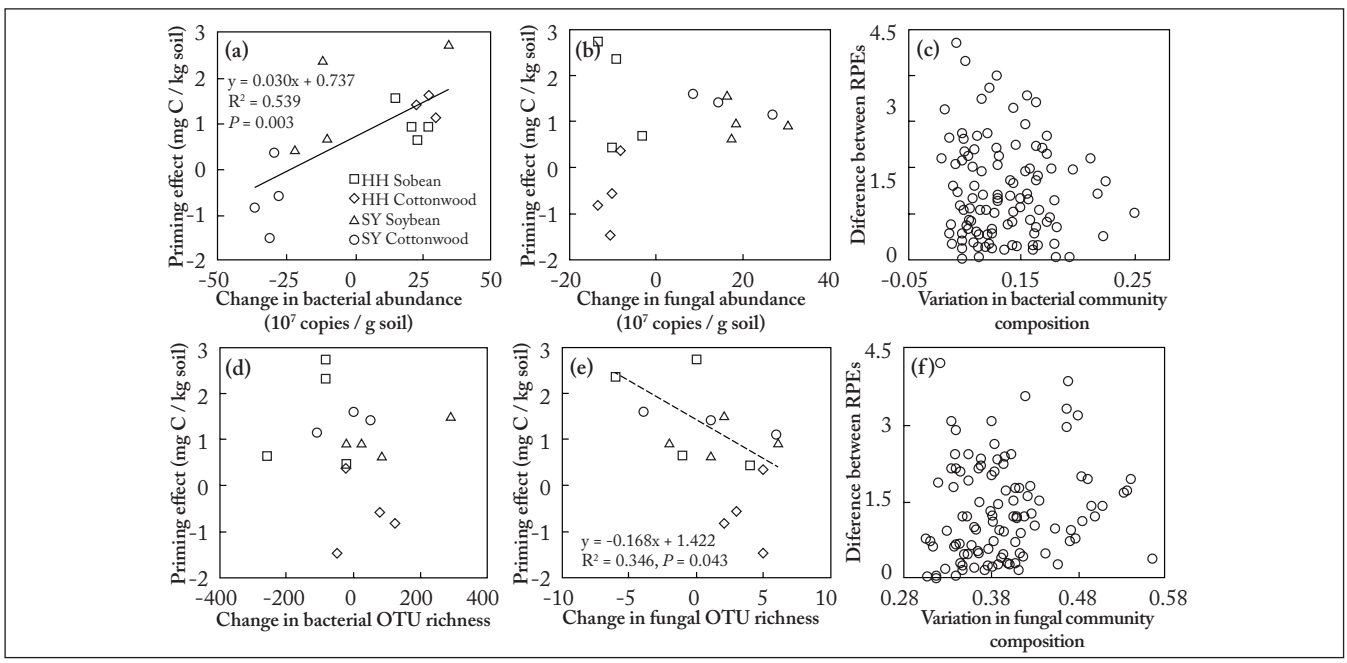

Fig. 3. Relationships between the rhizosphere priming effect and the changes in the abundance, richness and composition of soil bacterial/fungal communities. Only the significant results (P<0.05) are shown in the figure. Mantel test revealed that there was no significant relationship (P>0.05) between the rhizosphere priming effect and the bacterial/fungal community composition variation.

Fig. 3. Relaciones entre el efecto de preparación de la rizósfera y los cambios en la abundancia, riqueza y composición de comunidades de bacterias y hongos en el suelo. Solo se muestran los resultados significativos (P<0,05) en la figura. El test Mantel reveló que no hubo diferencia significativa (P>0,05) entre el efecto de preparación de la rizósfera y la variación en la composición de las comunidades de hongos y bacterias.

RPE also showed a significant correlation with the changes in fungi richness (Fig. 3e), the slope was negative, meaning that this relationship was illogical and that it happened just by chance.

We further adopted a structural equation model to analyze the effect of plant species and experimental locations on RPE, with all the microbial variables and soil physicochemical condition as the potential explanatory variables (see Supporting Method; Table 1; Fig. 4). Although some other indexes such as fungi abundance and soil physicochemical condition were also found to explain part of the variation in RPE, soil bacteria abundance was found to be the one with the largest explanatory power (with the largest value of standardized path coefficient of 0.98; Fig. 4). Overall, the results were consistent with those from correlation analysis (Fig. 3), confirming that the abundance of soil bacterial communities was the

Table 1. Results of structural equation modeling of plant species and experimental locations on rhizosphere priming effect as illustrated in Figure 2.
Tabla 1. Resultados del modelado de la ecuación estructural de las especies vegetales y sitios experimentales en el efecto de preparación de la rizósfera como se ilustra en la Figura 2.

			Estimate	S.E.	C.R.	P
Soil physicochemical indexes	<--	Plant species	-0.397	0.034	-11.74	***
Soil physicochemical indexes	<--	Location	0.098	0.034	2.896	0
Bacteria abundance	<--	Plant species	5.981	12.131	0.493	0.62
Bacteria richness	<--	Plant species	-108.74	90.033	-1.208	0.23
Bacteria composition	<--	Plant species	0.079	0.049	1.63	0.1
Fungi abundance	<--	Plant species	4.097	4.106	0.998	0.32
Fungi richness	<--	Plant species	3.305	2.861	1.155	0.25
Fungi composition	<--	Plant species	0.023	0.103	0.219	0.83
Bacteria abundance	<--	Soil physicochemical indexes	31.625	29.146	1.085	0.28
Bacteria richness	<--	Soil physicochemical indexes	-301.9	216.32	-1.396	0.16
Bacteria composition	<--	Soil physicochemical indexes	0.191	0.117	1.634	0.1
Fungi abundance	<--	Soil physicochemical indexes	13.816	9.866	1.4	0.16
Fungi richness	<--	Soil physicochemical indexes	5.715	6.874	0.831	0.41
Fungi composition	<--	Soil physicochemical indexes	0.001	0.247	0.004	1
Bacteria abundance	<--	Location	16.673	4.659	3.578	***
Bacteria richness	<--	Location	72.019	34.582	2.083	0.04
Bacteria composition	<--	Location	-0.03	0.019	-1.614	0.11
Fungi abundance	<--	Location	12.765	1.577	8.093	***
Fungi richness	<--	Location	-0.521	1.099	-0.474	0.64
Fungi composition	<--	Location	0.073	0.04	1.852	0.06
RPE	<--	e10	0.378	0.071	5.292	***
RPE	<--	Bacteria abundance	0.041	0.007	6.061	***
RPE	<--	Bacteria richness	0.001	0.001	0.585	0.56
RPE	<--	Bacteria composition	3.734	2.329	1.603	0.11
RPE	<--	Fungi abundance	-0.055	0.012	-4.736	***
RPE	<--	Fungi richness	-0.025	0.03	-0.824	0.41
RPE	<--	Fungi composition	3.074	0.848	3.625	***
RPE	<--	Soil physicochemical indexes	0.946	0.291	3.25	0

Given are the unstandardized path coefficients (estimates), standard error of regression weight (S.E.), the critical value for the regression weight (C.R.), and the level of significance for the regression weight (P). *** = P<0.001.
Se incluyen los coeficientes de senda no estandarizados (estimaciones), error estándar del peso de la regresión (S.E.), el valor crítico para el peso de la regresión (C.R.), y el nivel de significancia para el peso de la regresión (P). *** = P<0,001.

primary driver of RPE. Detailed analyses between RPE and the changes in relative abundance of dominant microbial phyla further suggested that the two phyla of Acidobacteria and Planctomycetes were the main RPE drivers (Table 2).

Table 2. Results of Pearson correlation between PRE and the changes in dominant bacterial taxonomic groups.
Tabla 2. Resultados de la correlación Pearson entre PRE y los cambios en los grupos taxonómicos de las bacterias dominantes.

Bacterial phyla	Average relative abundance (%)	Slope	R^2	P
Acidobacteria	11.852 (0.734)	15,763	0,392	**0,013**
Actinobacteria	17.592 (1.309)	-19,400	0,349	**0,020**
Bacteroidetes	9.307 (1.208)	-14,804	0,014	0,679
Chloroflexi	5.696 (0.362)	-22,731	0,088	0,282
Firmicutes	7.095 (0.929)	-12,693	0,119	0,207
Gemmatimonadetes	2.304 (0.186)	-37,659	0,029	0,542
Planctomycetes	6.598 (0.400)	37,880	0,294	0,037
Proteobacteria	31.668 (1.210)	-1,639	0,001	0,892

P values < 0.05 in bold.

Bacteria rather than fungi as the primary driver could be due to two reasons. First, bacteria and fungi could generally be classified as the r- and K-strategists, respectively (Dungait et al., 2013). In other words, bacteria are opportunists, and their abundance responds rapidly to the change in the resources supplied from plants. The rapid increase (or decrease) in their abundance induced by plants is very likely to cause them to decompose more (or less) SOM, resulting in the positive (or negative) RPE. In contrast, fungi are more insensitive to environmental changes than bacteria (DeAngelis et al., 2015). Second, bacteria are generally more abundant than fungi in many soils, and thus they are responsible for a large proportion of soil respiration and the relevant RPE. Meanwhile, the abundance rather than richness/composition of bacterial communities as the primary driver should be due to that the abundance was more sensitive to environmental changes than the richness/composition (Zhang & Han, 2012). For example, if the difference in attributes (e.g., fitness) among different species was very small, the richness/composition of a biological community will change little under environmental changes; in contrast, the community abundance may decrease/increase a lot under environmental changes, independent of species

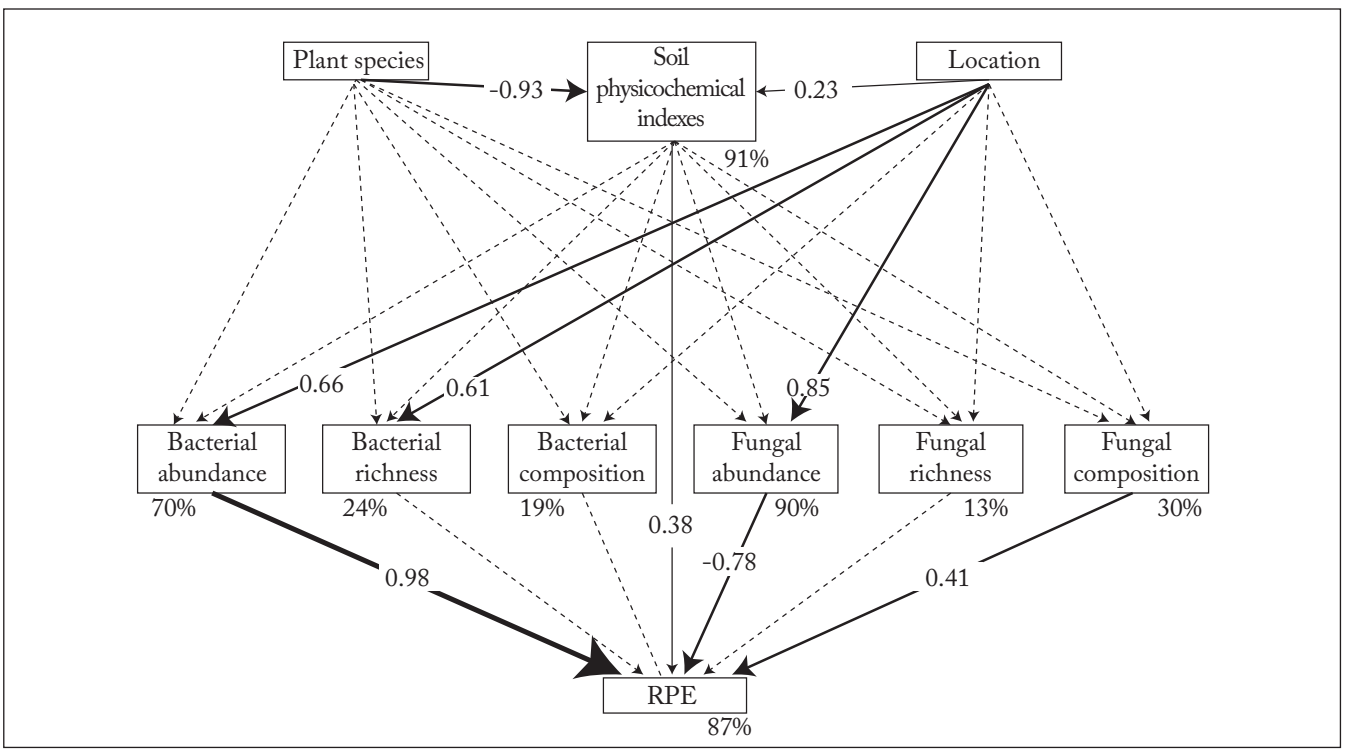

Fig. 4. Structural equation model analysis of the effect of plant species and experimental locations on RPE. The final model fit the data well: χ^2 = 24.588, P=0.104, df = 27, n = 15. Numbers at solid arrows (P<0.05) are standardized path coefficients (equivalent to correlation coefficients), and width of the arrows indicates the strength of the relationships. The dashed arrows indicate non-significant relationships (P>0.05). Percentages close to variables indicate the variance explained by the model (R^2).
Fig. 4. Análisis estructural del modelo de la ecuación del efecto de las especies vegetales y sitios experimentales en el efecto de preparación de la rizósfera (RPE). El modelo final ajustó bien los datos: χ^2 = 24,588, P=0,104, g.l. = 27, n = 15. Los números en las flechas sólidas (P<0,05) son coeficientes de senda estandarizados (equivalents a los coeficientes de correlación), y el ancho de las flechas indica la fortaleza de las relaciones. Las flechas cortadas indican relaciones no significativas (P>0,05). Los porcentajes cerca de las variables indicant la varianza explicada por el modelo (R^2).

differences (Kuske et al., 2002). Overall, the critical role of bacteria abundance in driving RPE suggests that it can be used as an indicator index in monitoring this process and in improving the terrestrial carbon cycling models.

REFERENCES

Cheng, W., W.J. Parton, M.A. Gonzalez-Meler, R. Phillips, S. Asao, G.G. McNickle, E. Brzostek & J.D. Jastrow (2014). Synthesis and modeling perspectives of rhizosphere priming. *New Phytologist* 201: 31-44.

DeAngelis, K.M., G. Pold, B.D. Topçuoğlu, L.T.A. van Diepen, R.M. Varney, J.L. Blanchard, J. Melillo & S.D. Frey (2015). Long-term forest soil warming alters microbial communities in temperate forest soils. *Frontiers in Microbiology* 6: 104.

Di Lonardo, D.P., W. De Boer, P.J.A. Klein Gunnewiek, S.E. Hannula & A. Van der Wal (2017). Priming of soil organic matter: Chemical structure of added compounds is more important than the energy content. *Soil Biology and Biochemistry* 108: 41-54.

Dungait, J.A.J., S.J. Kemmitt, L. Michallon, S. Guo, Q. Wen, P.C. Brookes & R.P. Evershed (2013). The variable response of soil microorganisms to trace concentrations of low molecular weight organic substrates of increasing complexity. *Soil Biology and Biochemistry* 64: 57-64.

Kent, A.D. & E.W. Triplett (2002). Microbial Communities and Their Interactions in Soil and Rhizosphere Ecosystems. *Annual Review of Microbiology* 56: 211-236.

Kuske, C.R., L.O. Ticknor, M.E. Miller, J.M. Dunbar, J.A. Davis, S.M. Barns & J. Belnap (2002). Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. *Applied and Environmental Microbiology* 68: 1854-1863.

Kuzyakov, Y. (2002). Factors affecting rhizosphere priming effects. *Journal of Plant Nutrition and Soil Science* 165: 382-396.

Kuzyakov, Y. & G. Domanski (2000). Carbon input by plants into the soil. Review. *Journal of Plant Nutrition and Soil Science* 163: 421-431.

Su, T., F.A. Dijkstra, P. Wang & W. Cheng (2017). Rhizosphere priming effects of soybean and cottonwood: do they vary with latitude? *Plant and Soil* 420: 349-360.

Warembourg, F.R., C. Roumet & F. Lafont (2003). Differences in rhizosphere carbon-partitioning among plant species of different families. *Plant and Soil* 256: 347-357.

Zhang, X. & X. Han (2012). Nitrogen deposition alters soil chemical properties and bacterial communities in the Inner Mongolia grassland. *Journal of Environmental Sciences* 24: 1483-1491.

Zhu, B. & W. Cheng (2011). Rhizosphere priming effect increases the temperature sensitivity of soil organic matter decomposition. *Global Change Biology* 17: 2172-2183.