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Short communication

Bacterial community shifts associated with high abundance of *Rhizobium* spp. in potato roots under macronutrient-deficient conditions

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ABSTRACT

We evaluated the impacts of a macronutrient deficiency on the community structure of root-associated bacteria in potato (*Solanum tuberosum*) cultivation. Potato plants were cultivated in a long-term experimental field under a nitrogen, phosphorus, or potassium deficiency, and roots were sampled at the early flowering stage. Amplicon libraries of 16S rRNA gene were constructed for root-associated bacteria, and bacterial diversity was analyzed by means of pyrosequencing. Statistical analyses showed significantly lower species evenness in the nutrient-deficient plots compared with the value in a plot with standard fertilization. Phylogenetic analyses revealed that the abundance of *Rhizobium* spp. increased dramatically in all plots under a macronutrient deficiency.

To date, numerous studies of root–microbe interactions have focused on rhizobia, mycorrhizal fungi, and their symbiotic plant partners. Researchers have also studied beneficial plant growth-promoting rhizobacteria (PGPR), and more than 20 genera of bacteria have been characterized as PGPR under laboratory conditions (Kloepper et al., 1980; Compant et al., 2010). However, applications of these PGPR are rarely effective in real agronomic practices. This is mainly thought to result from a lack of knowledge about the ecology and functions of these microorganisms under field conditions (Ikeda et al., 2010). Thus, we need more comprehensive knowledge of plant–microorganism interactions in realistic agronomic environments before we can efficiently begin to utilize these beneficial microorganisms (Normander and Prosser, 2000; Berg et al., 2005).

The quality and quantity of rhizodeposition (both shedding of root cells and production of root exudates) depend strongly on the availability of plant macronutrients such as N (Paterson and Sim, 2000), P (Ohwaki and Hirata, 1992), and K (Kraffczyk et al., 1984). These previous studies suggested that fertilization conditions are one of the main forces that shape the root and rhizosphere microbial community structure by changing rhizodeposition. The aims of the present study were to characterize the root-associated bacterial communities in potato (*Solanum tuberosum*) roots grown under macronutrient deficient conditions and to describe the ecological rules that govern the community structure of root-associated bacteria in these plants.

Seed tubers of cultivar 'Matilda' were planted in a plot with standard fertilization (NPK) and in plots that lacked supplemental fertilization with one of three macronutrients: PK, NK, and NP plots, which were deficient in N, P_2O_5 and K_2O, respectively. All plots were 31.2 m^2 in size, and planting was performed on 22 May 2012 in a long-term experimental field in Japan (42°89.2′ N, 143°07.7′ E, 93 m a.s.l.) that had been maintained under rotation of upland crops with potato, maize, sugar beet or soybean grown during the

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Table 1
Chemical characteristics of soil samples.

Fertilization plots[a]	pH (H_2O)	Organic C (%)	Total N (g kg^{-1})	Available N (mg kg^{-1})	NH_4–N (mg kg^{-1})	NO_3–N (mg kg^{-1})	Truog P (mg kg^{-1})	Ex-K_2O (mg kg^{-1})
NPK	5.29	5.22	2.48	54.0	9.4	8.9	111.7	115.4
NP	5.29	5.34	2.48	52.8	8.5	8.5	121.4	60.2
NK	5.29	5.50	2.54	43.6	10.8	11.5	35.5	190.9
PK	5.77	5.52	2.54	51.3	8.9	9.6	133.0	270.0

[a] A long-term experimental field consisted with NPK, NP, NK and PK plots. See the main text for details.

Table 2
Average dry weight and N, P and K content for shoots of potato plants.

Fertilization plots[a]	Dry weight (g $plant^{-1}$)	N (%)	P (%)	K (%)
NPK	20.61a	3.27c	0.20c	3.40a
NP	15.93ab	3.87bc	0.22b	2.80a
NK	19.15ab	4.67 ab	0.17d	3.17a
PK	7.00b	3.63c	0.34a	3.50a

Data were analyzed with ANOVA and Tukey's HSD test ($P \leq 0.05$%). Means ($n = 3$) followed by a common letter in a column are not significantly different.

[a] A long-term experimental field consisted with NPK, NP, NK and PK plots. See the main text for details.

summer and no cultivation during the winter since 1994 at the Memuro Research Station of the Hokkaido Agricultural Research Center (Memuro, Hokkaido, Japan). For potato cultivation, the NPK plot received 60, 200, and 120 kg of N, P_2O_5, and K_2O ha^{-1}, respectively. The NP, NK, and PK plots received similar fertilization, but without K, P, and N, respectively.

As potato plants do not take up large amounts of nutrients from the soil after flowering, and because relocation of nutrient elements among plant tissues and senescence occur after flowering, we chose the early flowering period for sampling of soil bacteria in order to detect the maximum impacts of the fertilizer regime on root-associated bacteria. The roots of nine plants at this stage were sampled from each plot on 12 July 2012. Roots were serially washed with tap water and sterilized water and were then stored at −30 °C until they were used for DNA extraction. Shoots of three plants were sampled in each plot, and their dry weight was measured after drying at 80 °C for 2 days. N, P, and K concentrations in the shoots and the general soil characteristics at the time of sampling were also determined for each plot by the Tokachi Nokyoren Agricultural Research Institute (Obihiro, Hokkaido, Japan). The soil type was classified as an Andosol. Table 1 summarizes the characteristics of the soil samples.

Approximately 20 g of roots from each plant were ground into powder in liquid nitrogen using a mortar and pestle. Root powder derived from three plants was combined and was processed as a single composite sample for the bacterial cell extraction (Ikeda et al., 2009). To eliminate plant DNA, enriched bacterial cells from a composite sample were treated with DNase I (Takara Bio, Otsu, Japan) in 50 μL of solution containing 2 μL of DNase I (10 U) and 10 × buffer at 37 °C for 20 min according to the manufacturer's

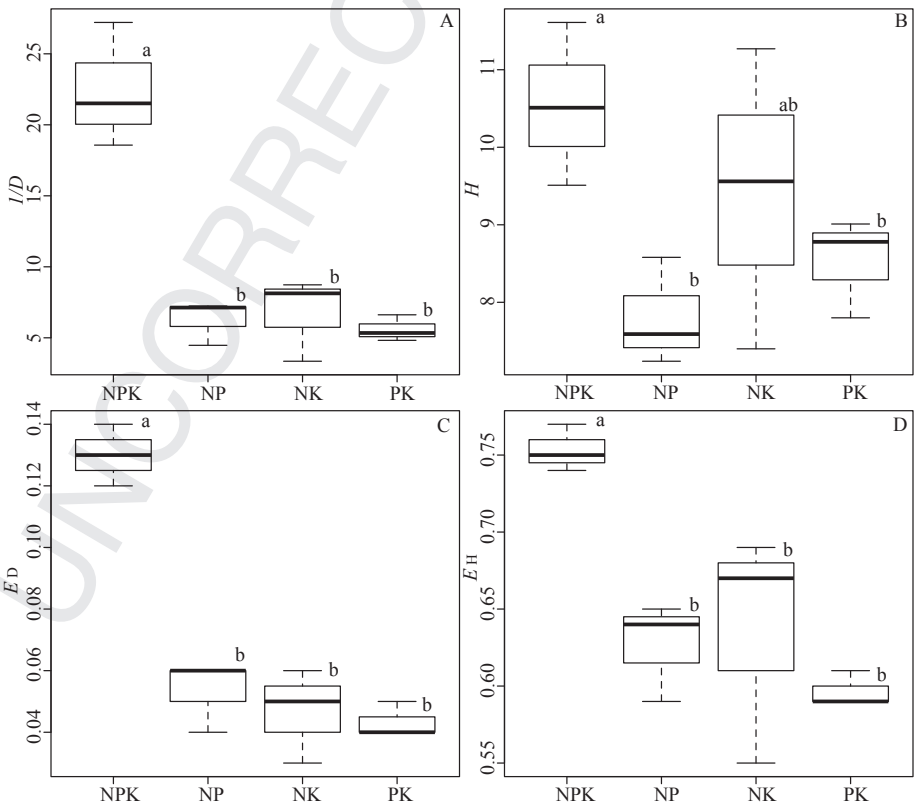

Fig. 1. Box plots for species diversity indices: (A) Simpson's diversity ($1/D$), (B) Shannon's diversity (H'), (C) Simpson's evenness (E_D), and (D) Shannon's evenness (E_H). Values are based on the 16S rRNA gene sequencing data (Supplemental Table S2). Values in a graph labeled with the same letter did not differ significantly (Tukey's HSD multiple comparison test, $p < 0.05$).

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