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Effects of mowing dominant grasses on root exudation and soil nitrogen cycling in a natural sod culture apple orchard

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Abstract: We evaluated the effects of mowing dominant grasses on root exudation and soil nitrogen (N) cycling by assessing metabolomics analysis of root exudates, microbial metabolism, the abundance of N-cycling-related prokaryotes, and different forms of N concentrations in soil. The treatments included *Polygonum aviculare* L. mowing (T1), *Digitaria sanguinalis* (L.) Scop. mowing (T2), and no mowing as the controls (CK1 and CK2). The results showed that compared with the no mowing control (CK1 and CK2), T1 and T2 root exudates contained 223 (178 up-regulated, 45 down-regulated) and 183 (40 up-regulated, 143 down-regulated) differential metabolites, respectively. The average well colour development (AWCD) could reflect the microbial metabolic activity. The AWCD values of T1 were increased while that of T2 decreased on the 2nd day after mowing. The variation in root exudates was the main reason for the change in soil AWCD values and carbon utilisation of T1 and T2 on the 2nd day after mowing. Mowing increased soil microbial biomass N content significantly in the T1 and T2 topsoil. The NO_3^--N and NH_4^+-N contents in the 0–10 cm soil increased on the 2nd day after T1 mowing with an increase in the nitrogenase iron protein gene (*nif*H), glutamate dehydrogenase gene (*gdh*), ammonia monooxygenase gene (*amo*A) of ammonia-oxidising archaea (AOA) and ammonia-oxidising bacteria (AOB) abundance. However, NO_3^--N content decreased on the 2nd day after T2 mowing following a decrease in AOA-*amo*A and AOB-*amo*A gene abundance. The results of this study will facilitate the optimisation of sod culture orchard N management, reduction of N fertiliser input, and improvement of N utilisation efficiency.

Keywords: orchard cover cultivation; mowing management; root exudates component; soil nitrogen transformation; soil microbial metabolism

Traditional orchard soil management in China predominately uses tillage practices to clear groundcover, which can rapidly reduce soil organic matter (SOM) and destroy soil structure. Orchard sod culture is a soil cover cultivation technique to grow grass in the whole orchard or inter-rows; as an extensively applied soil management technique globally, it has numerous advantages, such as increased SOM content, improved soil microbial community diversity and orchard ecosystem diversity. Some studies found that higher plant diversity was associated with higher microbial diversity (Eisenhauer et al. 2013) and that microbial diversity could be increased by adding a diverse exudate mix to plant monocultures (Steinauer et al. 2016). Species diversity also affects soil nutrient transformation, and genes related to different stages of nitrogen (N) cycling are affected by plant selection (Schmidt et al. 2019). For example, the maize

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rhizosphere is enriched by functional genes related to N fixation, nitrification, and denitrification (Ai et al. 2013, Li et al. 2014, Wang et al. 2017). This effect was also observed in treatments with artificial maize root exudates (Henry et al. 2008), implying that exudates are the major factors influencing microbial N cycling.

Grass mowing is the main management technique in a sod culture apple orchard. The effects of mowing and mulching inter-row grasses on soil properties, nutrient content, and microbial community structures have been widely studied (Gómez et al. 2009, Zhou et al. 2019). However, the influence of mowing on soil N cycling remains unclear, especially in natural sod culture orchards with a cultivar of grass species. To understand the effect of mowing dominant grasses on soil N cycling, reduce N fertiliser inputs, and improve N utilisation efficiency in apple orchards, we carried out field mowing and hydroponic experiments to examine the influence of mowing on changes in root exudate components and microbial metabolism, the abundance of N-cycling-related functional genes, such as the nitrogenase iron protein gene (*nif*H) associated with nitrogen fixation, the glutamate dehydrogenase gene (*gdh*) associated with ammoniation, and the ammonia monooxygenase gene (*amo*A) associated with nitrification, and the concentrations of different forms of soil N during a critical period when apple trees require fertiliser.

MATERIAL AND METHODS

Study site and plant cultivation. Experiments were conducted in a 12-year-old apple orchard at Shenyang Agricultural University (41°83'N, 123°56'E, 76.2 m a.s.l.), which has been an experimental interplanting site for a long time. The area was 1 ha. The soil in the orchard was classified as brown earth (Hapli-Udic Cambisol) according to FAO classification and has properties: clay loam, 0.86% SOM (potassium dichromate oxidation – ferrous sulfate titration method), 49.8 mg/kg alkali hydrolysable N (alkali diffusion method, 1 mol/L sodium hydroxide diffusion – 20 g/L boric acid absorption – 0.005 mol/L sulfuric acid titration), 35.6 mg/kg available phosphorus (Olsen method, 0.5 mol/L sodium bicarbonate extraction – molybdenum antimony resistance colorimetric), 76.2 mg/kg available potassium (1 mol/L ammonium acetate extraction – flame photometric method). Apple trees (cv. Hanfu/GM256/*Malus baccata* Borkh.) were planted in a south-to-north direction at a density of 2.0 m × 4.0 m, in a ridge cultivation system (1.0 m wide, 20.0 cm high). Natural sod culture has been implemented since 2009.

After years of artificial selection, a stable grassland community dominated by natural native grass species has been formed in the orchard. And then, an annual succession occurred in the dominant grass species. In our study, *Polygonum aviculare* L. and *Digitaria sanguinalis* (L.) Scop. (Crabgrass) were selected as the research subjects at the stage of the rapid growth of young fruit and shoots period (1–20 June 2018), rapid fruit expansion and flower differentiation period (20 July 2018–10 August 2018), which were critical periods when apple trees require fertiliser, respectively.

Field mowing experiment. There were four treatments: T1 (*P. aviculare* mowing); CK1 (*P. aviculare* no mowing); T2 (Crabgrass mowing), and CK2 (Crabgrass no mowing). Thirty-six plots were randomly selected from the orchard inter-rows with a uniform growth of *P. aviculare* and separated from the surrounding soil with PVC pipes (30 cm diameter, 30 cm height) on 1 May 2018. They were divided into two groups on average, the mowing group and the no mowing control group. Among the 18 plots in the mowing group, 15 plots were mowed on 1 June 2018, to a height of 20 cm, the remained 3 plots were not mowed. Another 36 uniform growth plots of Crabgrass were randomly selected on 20 June 2018 and mowed on 20 July 2018; the treatments were as same as *P. aviculare*. The experimental materials (0–10 cm soil, grass roots and leaves) of each treatment were sampled on 0 (no mowing), 2, 4, 6, 8, and 10 days after mowing with three replicates. The soils were used for the analysis of the content of soil microbial biomass N (SMB_N), NO_3^--N, and NH_4^+-N, the activity of microbial metabolism, and gene abundance of *nif*H, *gdh*, *amo*A of ammonia-oxidising archaea (AOA) and ammonia-oxidising bacteria (AOB). The grass roots and leaves were sampled for the analysis of root activity, nitrate reductase (NR) and glutamine synthetase (GS) activity.

Hydroponic experiment for root exudates. We carefully dug up 90 uniform growth of *P. aviculare* and Crabgrass plants from the orchard inter-rows on 1 June 2018 and 20 July 2018, respectively. The roots were rinsed with deionised water and then cultured in 6 plastic buckets (15 plants per bucket) with 1 L of deionised water, 3 of the buckets were mowed immediately. The grasses were incubated for 24 h. The culture medium was collected every 6 h and then subcultured with 1 L of fresh deionised

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water and stored in a plastic bottle at 4 °C. The roots of the four treatments were dried and weighed after four collections. The culture medium was mixed and filter-sterilised using a 0.22 μm filter (MEC, 50 mm). The ratio of the root dry weight and culture medium volume was determined for each treatment group, and the culture medium of the control was prepared in the same ratio. The culture medium (2 L) from each treatment was concentrated to 100 mL at 40 °C to determine the changes in the root exudates.

Measurement of different forms of soil nitrogen concentrations and activity of enzymes involved in nitrogen metabolism. SMB_N was determined using the chloroform fumigation-K_2SO_4 extraction method (Lin 2010). The soil NO_3^--N and NH_4^+-N contents were extracted from fresh soil samples with 0.01 mol/L $CaCl_2$ (soil and extractant ratio 1:2, extraction time 1 h) and detected using a continuous flow injection analyser (AA3-HR, SEAL, Norderstedt, Germany). Root activity was determined using the triphenyltetrazolium chloride (TTC) method (Zou 2006). The activity of NR and GS was determined using the sulfanilamide colorimetry and the $FeCl_3$ complexation colorimetric method, respectively (Zou 2006).

Root exudate metabolomics profiling. Metabolomics profiling was performed using a UPLC-ESI-Q-TOF-MS system (UHPLC, 1290 Infinity LC, Agilent Technologies, Santa Clara, USA) coupled with TripleTOF 5600 (AB Sciex, Framingham, USA). XCMS software (AB Sciex LLC, San Diego, USA) was then used for peak extraction and metabolite identification of the data, and SIMCA software (version 14.0, Umetrics, Umeå, Sweden) was used for multi-dimensional statistical analysis of the mass spectrum data. In this experiment, multi-dimensional statistical analysis VIP > 1 and univariate statistical analysis P-value < 0.05 were selected as metabolites with significant differences, while VIP > 1 and 0.05 < P-value < 0.1 were regarded as differential metabolites. And among the differential metabolites, FC (fold change analysis) > 1 was up-regulated, and FC < 1 was down-regulated.

Analysis of microbial metabolism. The Biolog Eco Plates™ assay was used to analyse microbial metabolism (Garland 1997). The average well colour development (AWCD) for plates was calculated as the mean of the blanked absorbance values for all 95 response wells per reading time. AWCD was used to describe the microbial metabolism activity, which could evaluate the carbon source utilisation capacity of microorganisms. Three diversity indices, Shannon, Simpson, and McIntosh, were used to calculate soil microbial carbon source utilisation diversity and evaluate species richness, the dominance of common species and uniformity in the microflora, respectively.

Analysis of nitrogen-cycling-related function genes abundance. Soil microbial DNA was used as a template for PCR amplification of *nif*H, *gdh*, and *amo*A. The gel blocks containing target genes were retrieved and sequenced as described by Li et al. (2018). Standard curves were obtained using serial dilutions of plasmids with known copy numbers and containing the *nif*H, *gdh*, and *amo*A gene fragments. All samples were analysed in triplicate.

Statistical analysis. Excel 2016 (Microsoft, Redmond, USA) was used for data fitting, analysis, and visualisation. Data were statistically analysed in Statgraphics (STN; St. Louis, USA). All data were first tested for normality. One-way ANOVA followed by the least significant difference (*LSD*) test to evaluate significant interactions. Differences between treatment means were considered significant at $P < 0.05$. Data represent means ± standard error (SE) of three biological replicates.

RESULTS AND DISCUSSION

Competition for nutrients between the inter-row grasses and apples was the main reason for hindering the promotion of sod culture techniques in China. Mowing, which was the main management technique in sod culture apple orchards, could alleviate

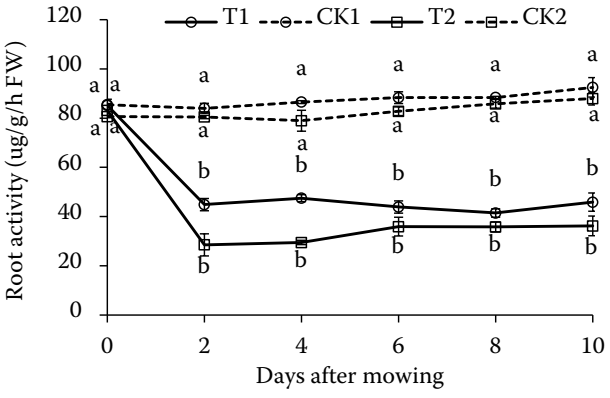

Figure 1. Root activity of mowing and no mowing *Polygonum aviculare* and Crabgrass. The data are means ± standard error (n = 3). Different letters indicate significant differences at $P < 0.05$ for the same grass at the same time. T1 – *P. aviculare* mowing; CK1 – *P. aviculare* no mowing; T2 – Crabgrass mowing; T2 – Crabgrass no mowing; FW – fresh weight

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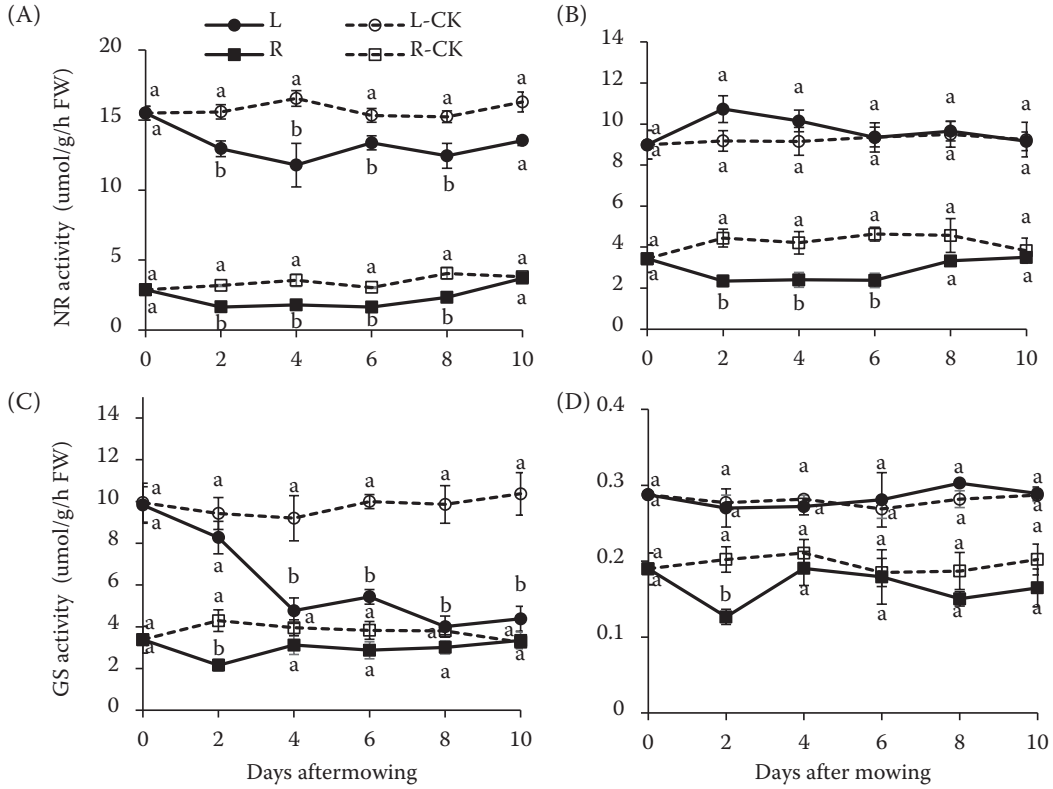

Figure 2. Nitrate reductase (NR) and glutamine synthetase (GS) activity in the roots and leaves of mowing and no mowing *Polygonum aviculare* and Crabgrass. (A) NR activity of *P. aviculare*; (B) NR activity of Crabgrass; (C) GS activity of *P. aviculare*, and (D) GS activity of Crabgrass. The data are means ± standard error (n = 3). Different letters indicate significant differences at $P < 0.05$ for the same grass at the same time. L – leaves; R – roots; CK – no mowing; FW – fresh weight

N competition by inhibiting the root activity and NR and GS activity of T1 and T2 (Figures 1 and 2). Mowing reduced the nutrient absorption capacity of inter-row dominant grasses and ensured the priority supply of soil nutrients to apple trees.

Effect of mowing on root exudate compositions. Mowing can stimulate the secretion of root exudates in the short term (Hokka et al. 2004, Paterson et al. 2005, Hamilton et al. 2008). In our study compared with the no mowing control (CK1 and CK2), T1 and T2 root exudates contained 223 (178 up-regulated, 45 down-regulated) and 183 (40 up-regulated, 143 down-regulated) differential metabolites after mowing, respectively (Figure 3). Root exudates play a key role in soil nutrient cycling and have a strong relationship with the specificity of species (Shen et al. 2020). The metabolites in the root exudates of T1 after mowing were increased, while those in the root exudates of T2 were decreased, resulting in different influences on the soil microbial community structures. The chemically diverse constituents of root exudates enrich specific microorganisms by stimulating their growth and/or by inducing or repressing specific microbial functions that play important roles in plant-microbe and microbe-microbe interactions (Doornbos et al. 2012, Huang et al. 2014, Carvalhais et al. 2015).

Effect of mowing on microbial metabolism and community structure. The AWCD value can reflect

Figure 3. Differential metabolites between mowing and no mowing of *Polygonum aviculare* and Crabgrass root exudates. The red part was up-regulated, while the blue part was relatively down-regulated. (A) heatmap for differential negative metabolites between mowing and no mowing of *P. aviculare*; (B) heatmap for differential positive metabolites between mowing and no mowing of *P. aviculare*; (C) heatmap for differential negative metabolites between mowing and no mowing of Crabgrass, and (D) heatmap for differential positive metabolites between mowing and no mowing of Crabgrass; T1 – *P. aviculare* mowing; T2 – Crabgrass mowing; CK1 – *P. aviculare* no mowing; CK2 – Crabgrass no mowing. The figures are on the following pages

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Figure 3A.

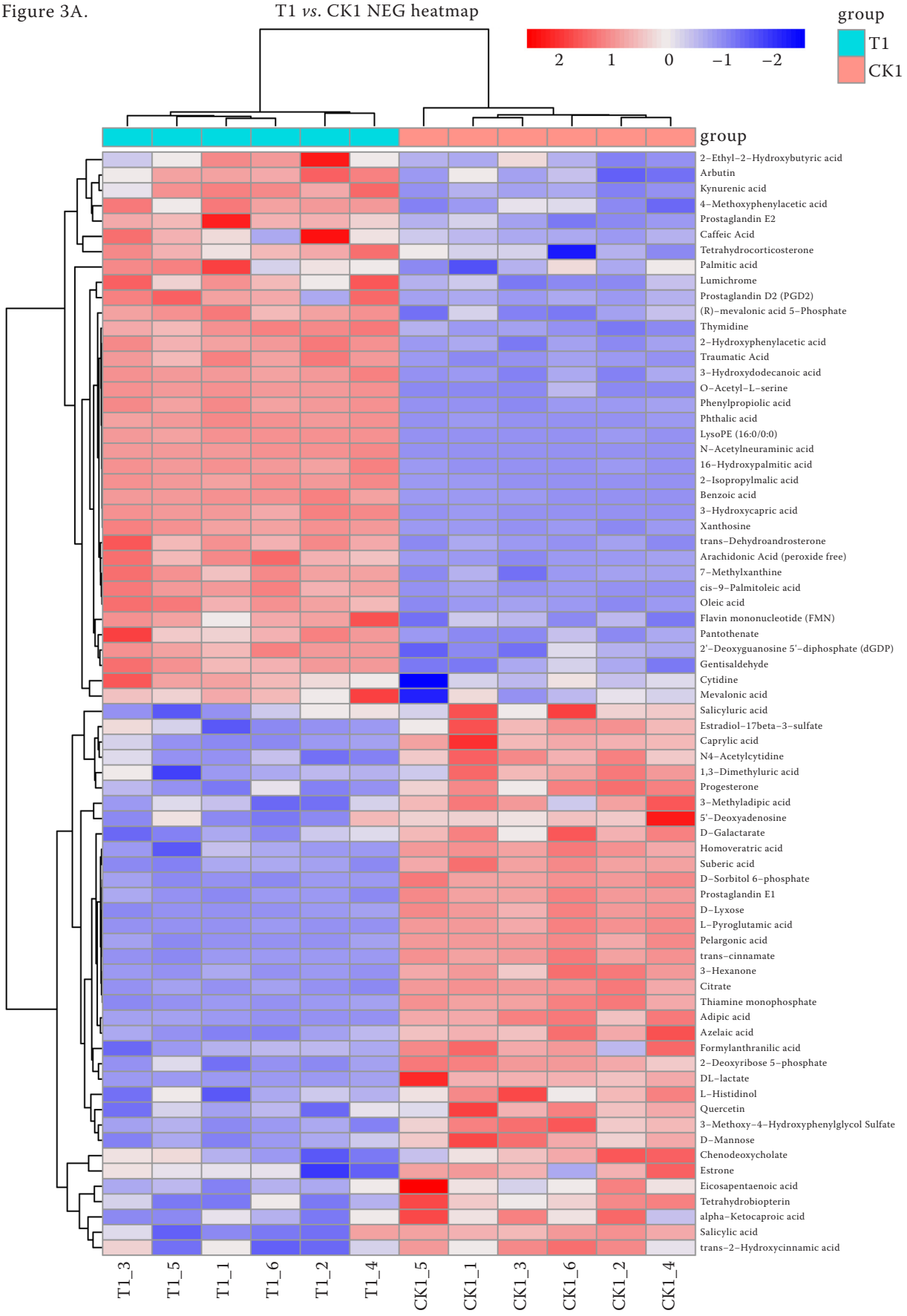

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Figure 3B. T1 *vs.* CK1 POS heatmap

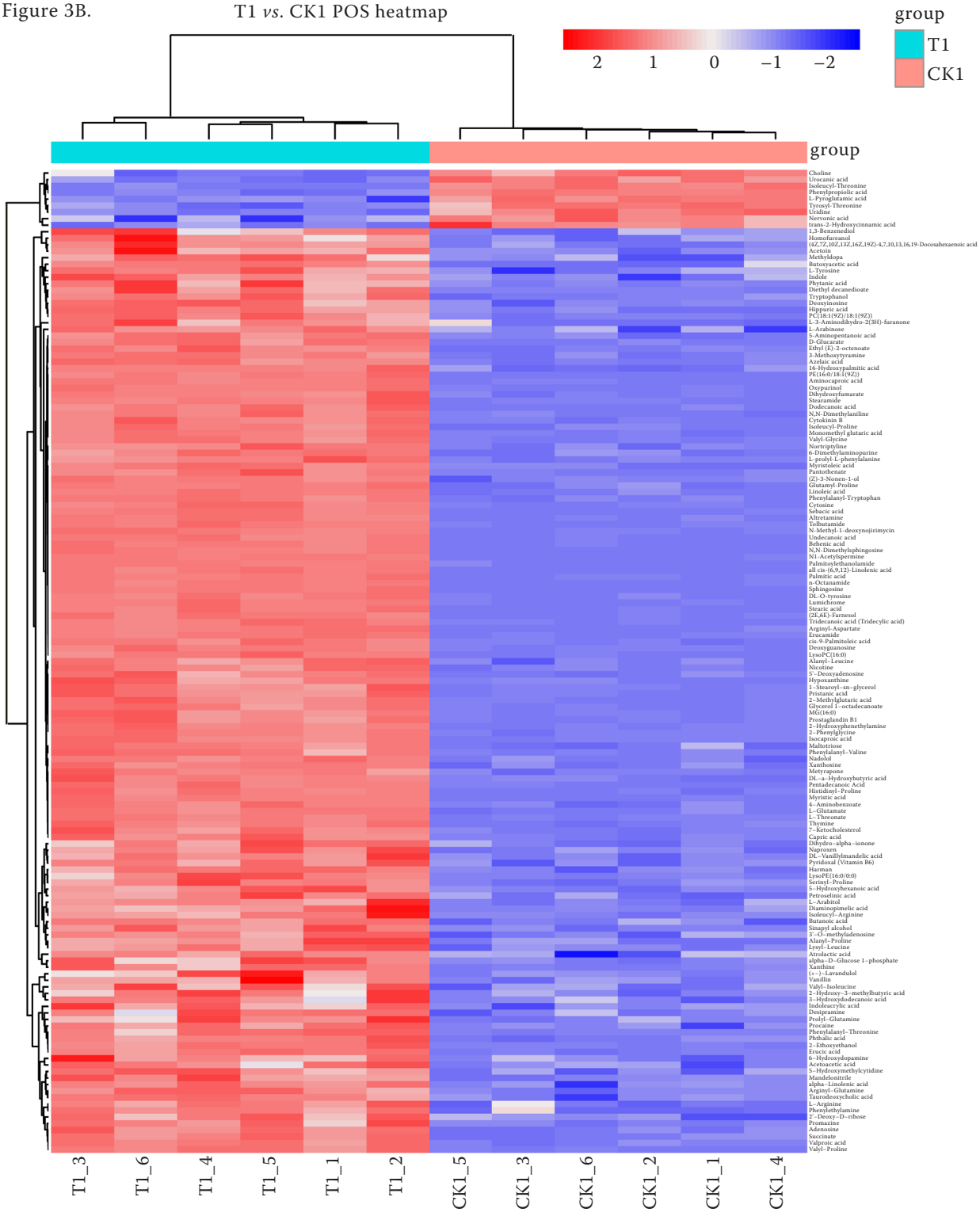

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Figure 3C. T2 *vs.* CK2 NEG heatmap

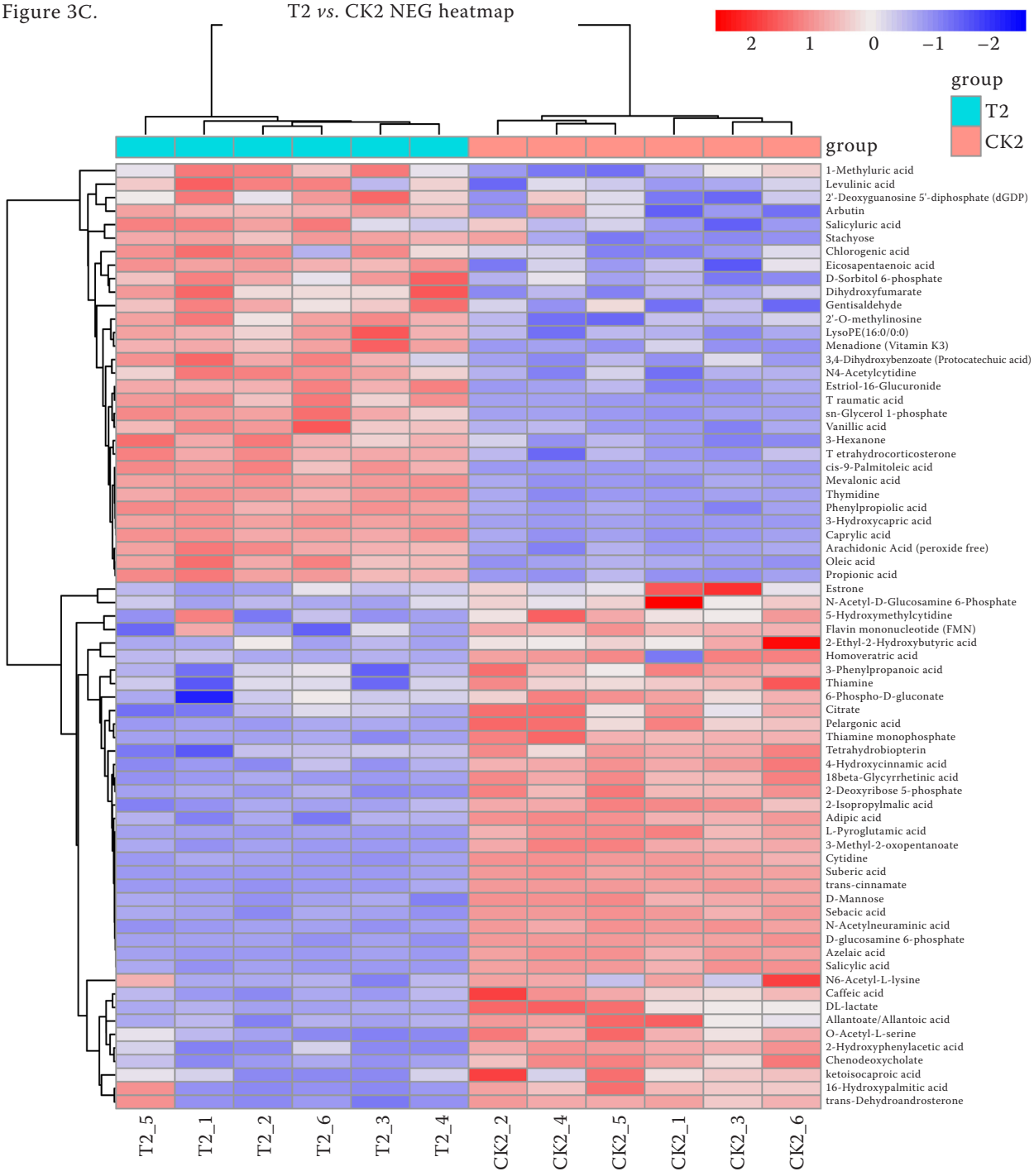

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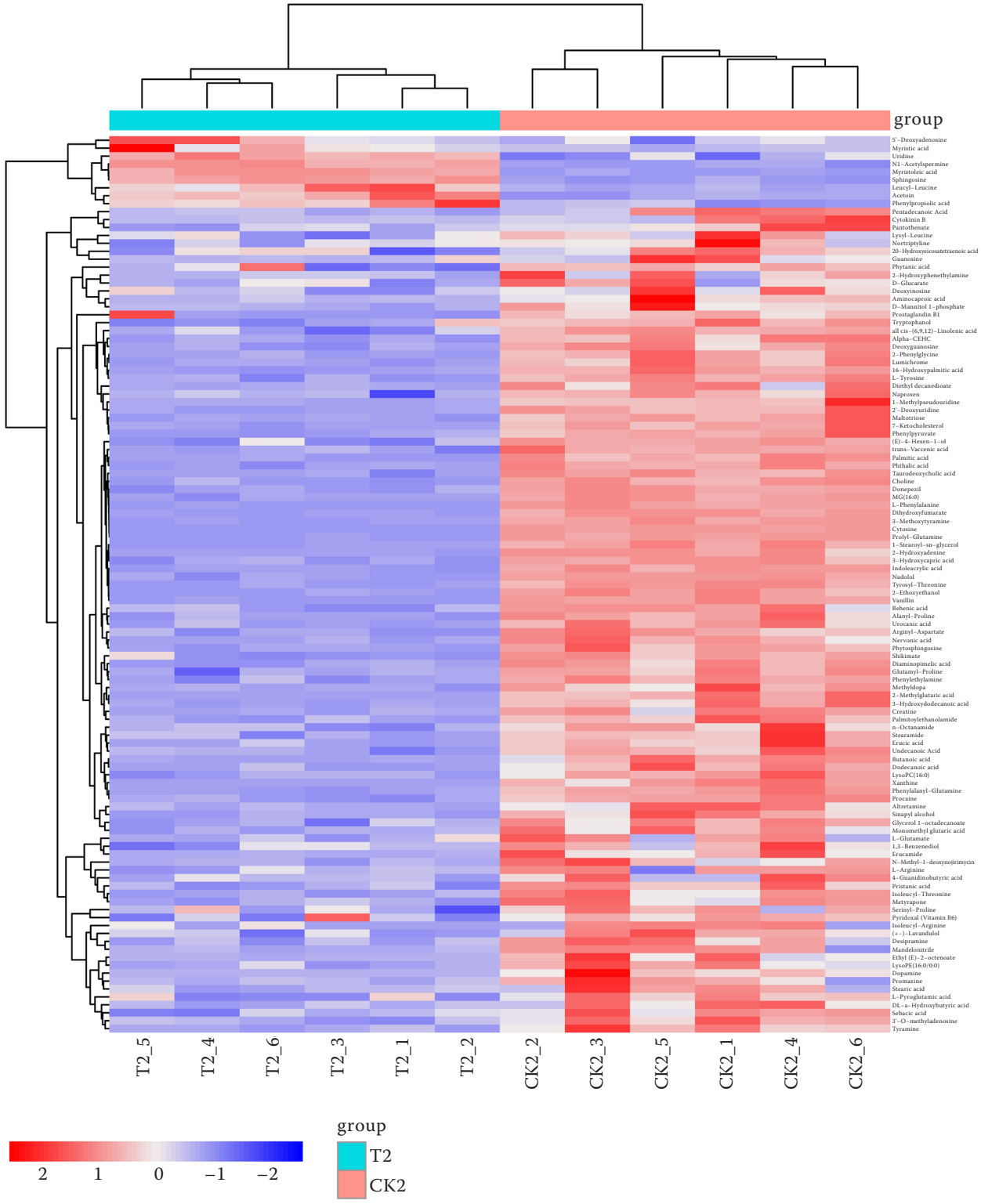
Figure 3D. T2 *vs.* CK2 POS heatmap



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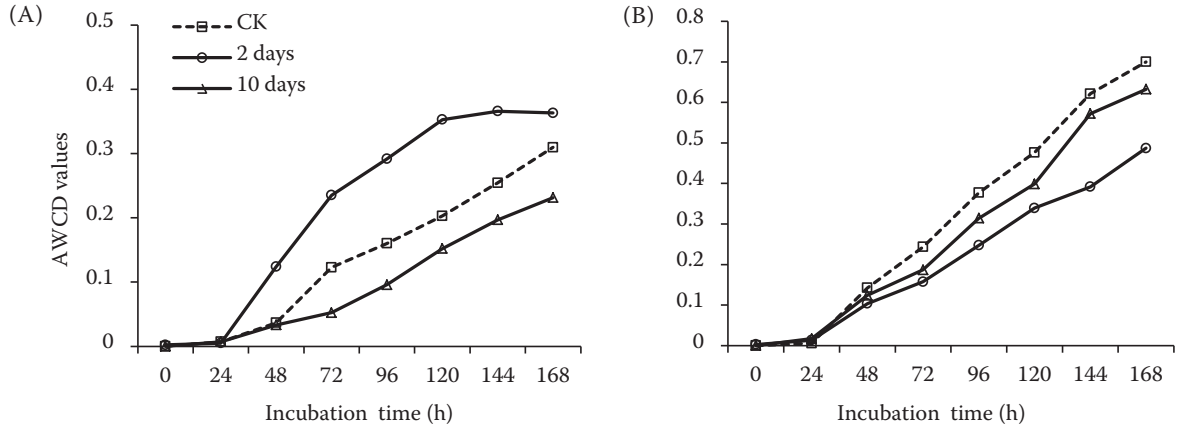

Figure 4. Dynamic changes in average well colour development (AWCD) values of *Polygonum aviculare* and Crabgrass in field soil microorganism after mowing. (A) AWCD values of *P. aviculare* soil microorganism, and (B) AWCD values of Crabgrass soil microorganism. CK – 0 day control (no mowing)

the soil microbial metabolic activities (Garland 1997). The increase in the composition of T1 root exudates promoted the metabolic activity of the soil microorganisms (AWCD values increased) and significantly increased the richness and evenness of species on the 2nd day after mowing (Figure 4A, Table 1). Soil microorganisms significantly increased the utilisation of carboxylic acids (62.36%), polymers (26.17%), phenolic acids (77.02%), and amines (96.15%) but significantly decreased the utilisation of carbohydrates (10.42%) and amino acids (8.91%) on the 2nd day after mowing (Figure 5A).

The decrease in the composition of T2 root exudates inhibited the metabolic activity of the soil microorganisms (AWCD values decreased) and decreased the species richness and dominance of the common species on the 2nd day after mowing (Figure 4B, Table 1). Soil microorganisms significantly increased the utilisation of amino acids (54.39%) and phenolic acids (84.01%) and significantly decreased levels of carbohydrates (35.25%), carboxylic acids (44.70%), and polymers (36.60%) on the 2nd day after mowing (Figure 5B). The change in soil microbial carbon (C) source utilisation was directly related to changes in root exudate composition (Figures 3 and 5).

The AWCD values of T1 decreased, and T2 increased on the 10th day after mowing, and both were lower than the control (Figure 4). It can be associated with the root architecture and recovery growth of the two grasses. C storage in the roots is essential for plant development or recovery when photosynthesis is limited (Schmitt et al. 2013). Such stored C is important for shoot regrowth after mowing so that the C input into the rhizosphere decreases. Mowing-induced reduction in root growth is also a consequence of the allocation of assimilates to support shoot regrowth (Guitian and Bardgett 2000). With the regrowth of the dominant grasses after mowing, the exudates released by the roots into the soil were gradually decreased.

Effect of mowing on soil nitrogen cycling and different forms of soil nitrogen concentrations. The diversity

Table 1. Soil microorganism diversity indices of different dominant grasses after mowing

Species	Treatment	Shannon index	Simpson index	McIntosh evenness index
Polygonum aviculare	CK	3.167 ± 0.019^b	0.987 ± 0.005^a	0.930 ± 0.012^b
	2 days	3.261 ± 0.026^a	0.939 ± 0.010^b	0.941 ± 0.014^a
	10 days	2.911 ± 0.013^c	0.975 ± 0.006^a	0.924 ± 0.005^b
Crabgrass	CK	2.998 ± 0.031^a	0.959 ± 0.002^a	1.047 ± 0.034^a
	2 days	2.914 ± 0.029^b	0.912 ± 0.005^b	1.036 ± 0.016^a
	10 days	3.037 ± 0.033^a	0.948 ± 0.016^a	1.012 ± 0.018^b

Data are means ± standard error (n = 3). Different letters indicate significant differences at $P < 0.05$ for the same grass. CK – 0 day (no mowing)

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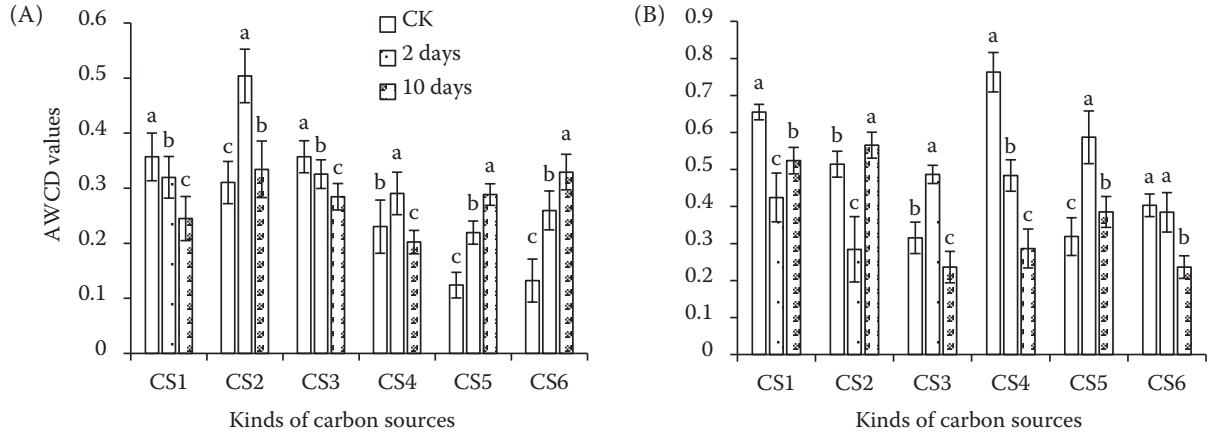

Figure 5. Analysis of microbial carbon (C) source utilisation in *Polygonum aviculare* and Crabgrass in field soil after mowing. (A) C source utilisation of *P. aviculare* soil microorganism, and (B) C source utilisation of Crabgrass soil microorganism. Data are means ± standard error (n = 3). Different letters indicate significant differences at $P < 0.05$ for the same carbon source at different times. CS1 – carbohydrates; CS2 – carboxylic acids; CS3 – amino acids; CS4 – polymers; CS5 – phenolic acids; CS6 – amines; CK – 0 day control (no mowing)

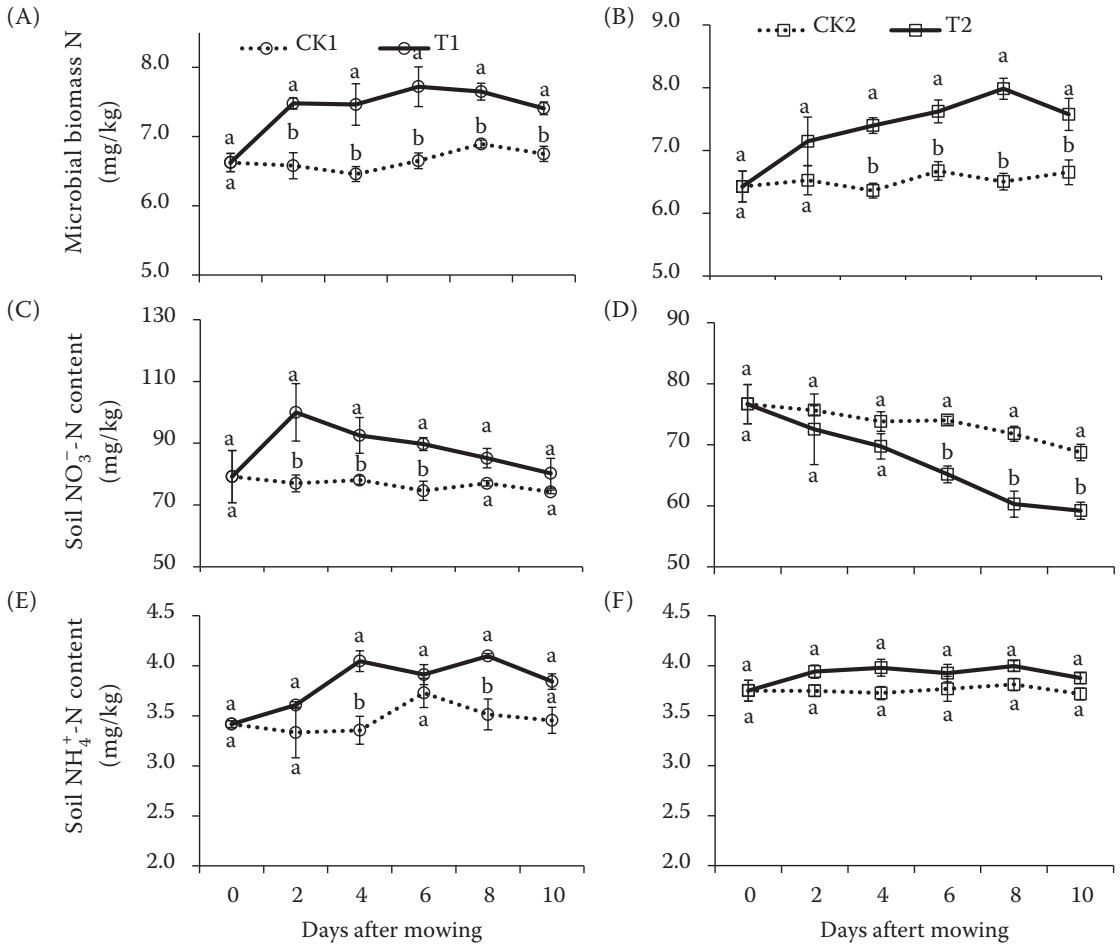

Figure 6. The different forms of soil nitrogen (N) content in *Polygonum aviculare* and Crabgrass mowing and no mowing field soil. (A) soil microbial biomass N (SMB_N) content of *P. aviculare* field soil; (B) SMB_N content of Crabgrass field soil; (C) NO_3^--N content of *P. aviculare* field soil; (D) NO_3^--N content of Crabgrass field soil; (E) NH_4^+-N content of *P. aviculare* field soil, and (F) NH_4^+-N content of Crabgrass field soil. Data are means ± standard error (n = 3). Different letters indicate significant differences at $P < 0.05$ between different treatments at the same time

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of soil microbial communities can intensify the availability of many essential plant nutrients by motivating the transformation of soil nutrients (Castrillo et al. 2017). Mowing provided a sufficient C source for the growth of soil microorganisms and significantly increased the SMB_N content in the 0 cm to 10 cm of T1 and T2 field soil. It reached a maximum of 7.72 mg/kg on the 6th day and 7.98 mg/kg on the 8th day, respectively. Due to the relatively stable composition of root exudates in the no mowing field soil, the soil microbial community structure was not changed (Figure 6A, B).

Soil microorganisms drive the morphological transformations of soil N (Kuypers et al. 2018). The abundance of N-cycling-related prokaryotes was affected by the change of the root exudates composition. In the present study, mowing increased NO_3^--N (99.99 mg/kg) and NH_4^+-N contents (Figure 6C, E) in the 0–10 cm T1 soil layer on the 2nd day by improving N fixation (*nif*H), ammoniation (*gdh*), and nitrification (AOA-*amo*A and AOB-*amo*A) in soil (Figure 7A). The content of soil NO_3^--N and NH_4^+-N after T1 mowing was increased firstly and then decreased; the difference was not significant on the 10th day. In addition, the soil NO_3^--N content of T2 and CK2 was decreased gradually during the experimental period (Figure 6D). The rapid growth rate, large root absorption area (fibrous root system) and high NO_3^--N affinity gave the Crabgrass a higher ability to absorb NO_3^--N. With the continuous growth of Crabgrass, the content of NO_3^--N in the soil decreased. The soil NO_3^--N content of T2 was lower than that of CK2, and the difference was the most significant on the 6th day. On the one hand, the nitrification (AOA-*amo*A and AOB-*amo*A) of Crabgrass field soil was inhibited after mowing (Figure 7B), on the other hand, Crabgrass has high regeneration capacity, as the regrowth of Crabgrass, the competitive absorption ability to NO_3^--N was also enhanced. The soil NH_4^+-N content (Figure 6F) of T2 and CK2 has no significant difference. Root exudates can not only be used as C sources for microorganisms but also can directly affect the process of soil N cycling. For example, some biological nitrification inhibitors (linoleic acid, linolenic acid, methyl linoleate, methyl-*p*-coumarate and 1,9-decanediol (Sun et al. 2016) have been identified, and their effects and mechanisms of inhibition elaborated.

In summary, mowing significantly inhibited the root activity and N assimilation ability of the dominant inter-row grasses in a natural sod culture orchard in the short term and ensured the priority supply of soil nutrients to apple trees. Mowing also significantly altered the composition of root exudates which have a strong relationship with the specificity of grasses and, in turn, had different effects on soil N cycling. Mowing increased the SMB_N content in the 0 cm to 10 cm of *P. aviculare* and Crabgrass field soil, promoted the mineralisation of organic N and ensured adequate nutrient supply for apple trees.

Although the composition of root exudates was measured by hydroponics, it proved that mowing had a positive effect on the soil N cycling. Future studies should focus on screening and verifying

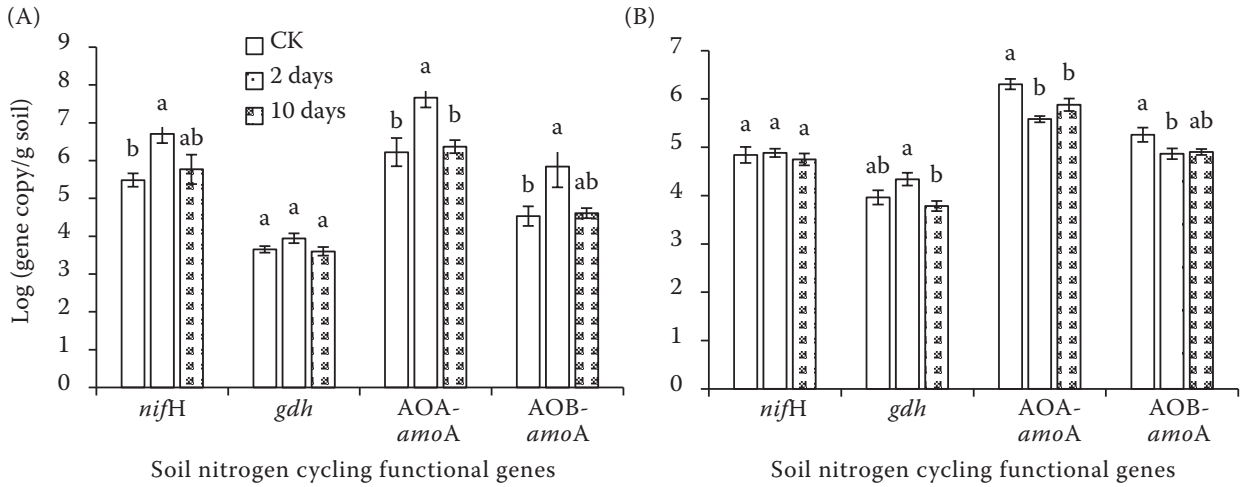

Figure 7. Analysis of soil nitrogen (N) cycling functional genes in *Polygonum aviculare* and Crabgrass field soil after mowing. (A) genes of *P. aviculare* soil, and (B) genes of Crabgrass soil. CK – 0 day control (no mowing). Data are means ± standard error (n = 3). Different letters indicate significant differences at $P < 0.05$ for the same gene at different times. *nif*H – nitrogenase iron protein gene; *gdh* – glutamate dehydrogenase gene; *amo*A – ammonia monooxygenase gene; AOA – ammonia-oxidising archaea; AOB – ammonia-oxidising bacteria

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functional metabolites that affect the soil N cycling in root exudates after mowing.

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REFERENCES

Ai C., Liang G.Q., Sun J.W., Wang X.B., He P., Zhou W. (2013): Different roles of rhizosphere effect and long-term fertilization in the activity and community structure of ammonia oxidizers in a calcareous fluvo-aquic soil. Soil Biology and Biochemistry, 57: 30–42.

Carvalhais L.C., Dennis P.G., Badri D.V., Kidd B.N., Vivanco J.M., Schenk P.M. (2015): Linking jasmonic acid signaling, root exudates, and rhizosphere microbiomes. Molecular Plant-Microbe Interactions, 28: 1049–1058.

Castrillo G., Teixeira P.J.P.L., Paredes S.H., Law T.F., de Lorenzo L., Feltcher M.E., Finkel O.M., Breakfield N.W., Mieczkowski P., Jones C.D., Paz-Ares J., Dangl J.L. (2017): Root microbiota drive direct integration of phosphate stress and immunity. Nature, 543: 513–518.

Doornbos R.F., van Loon L.C., Bakker P.A.H.M. (2012): Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere. A review. Agronomy for Sustainable Development, 32: 227–243.

Eisenhauer N., Dobies T., Cesarz S., Hobbie S.E., Meyer R.J., Worm K., Reich P.B. (2013): Plant diversity effects on soil food webs are stronger than those of elevated CO_2 and N deposition in a long-term grassland experiment. Proceedings of the National Academy of Sciences of the United States of America, 110: 6889–6894.

Garland J.L. (1997): Analysis and interpretation of community-level physiological profiles in microbial ecology. FEMS Microbiology Ecology, 24: 289–300.

Gómez J.A., Guzmán M.G., Giráldez J.V., Fereres E. (2009): The influence of cover crops and tillage on water and sediment yield, and on nutrient, and organic matter losses in an olive orchard on a sandy loam soil. Soil and Tillage Research, 106: 137–144.

Guitian R., Bardgett R.D. (2000): Plant and soil microbial responses to defoliation in temperate semi-natural grassland. Plant and Soil, 220: 271.

Hamilton III E.W., Frank D.A., Hinchey P.M., Murray T.R. (2008): Defoliation induces root exudation and triggers positive rhizospheric feedbacks in a temperate grassland. Soil Biology and Biochemistry, 40: 2865–2873.

Henry S., Texier S., Hallet S., Bru D., Dambreville C., Chèneby D., Bizouard F., Germon J.C., Philippot L. (2008): Disentangling the rhizosphere effect on nitrate reducers and denitrifiers: insight into the role of root exudates. Environmental Microbiology, 10: 3082–3092.

Hokka V., Mikola J., Vestberg M., Setälä H. (2004): Interactive effects of defoliation and an AM fungus on plants and soil organisms in experimental legume-grass communities. Oikos, 106: 73–84.

Huang X.F., Chaparro J.M., Reardon K.F., Zhang R.F., Shen Q.R., Vivanco J.M. (2014): Rhizosphere interactions: root exudates, microbes, and microbial communities. Botany, 92: 267–275.

Kuypers M.M.M., Marchant H.K., Kartal B. (2018): The microbial nitrogen-cycling network. Nature Reviews Microbiology, 16: 263–276.

Li M.C., Wei G.S., Shi W.C., Sun Z.T., Li H., Wang X.Y., Gao Z. (2018): Distinct distribution patterns of ammonia-oxidizing archaea and bacteria in sediment and water column of the Yellow River estuary. Scientific Reports, 8: 1584.

Li X.Z., Rui J.P., Xiong J.B., Li J.B., He Z.L., Zhou J.Z., Yannarell A.C., Mackie R.I. (2014): Functional potential of soil microbial communities in the maize rhizosphere. PLoS One, 9: e112609.

Lin X.G. (2010): Principles and Methods of Soil Microbiology Research. Beijing, Beijing Higher Education Press, 73–76.

Paterson E., Thornton B., Midwood A.J., Sim A. (2005): Defoliation alters the relative contributions of recent and non-recent assimilate to root exudation from *Festuca rubra*. Plant, Cell and Environment, 28: 1525–1533.

Schmidt J.E., Kent A.D., Brisson V.L., Gaudin A.C.M. (2019): Agricultural management and plant selection interactively affect the rhizosphere microbial community structure and nitrogen cycling. Microbiome, 7: 146.

Schmitt A., Pausch J., Kuzyakov Y. (2013): Effect of clipping and shading on C allocation and fluxes in soil under ryegrass and alfalfa estimated by ^{14}C labeling. Applied Soil Ecology, 64: 228–236.

Shen X., Yang F., Xiao C.W., Zhou Y. (2020): Increased contribution of root exudates to soil carbon input during grassland degradation. Soil Biology and Biochemistry, 146: 107817.

Steinauer K., Chatzinotas A., Eisenhauer N. (2016): Root exudate cocktails: the link between plant diversity and soil microorganisms? Ecology and Evolution, 6: 7387–7396.

Sun L., Lu Y.F., Yu F.W., Kronzucker H.J., Shi W.M. (2016): Biological nitrification inhibition by rice root exudates and its relationship with nitrogen-use efficiency. New Phytologist, 212: 646–656.

Wang C., Zheng M.M., Song W.F., Wen S.L., Wang B., Zhu C., Shen R. (2017): Impact of 25 years of inorganic fertilization on diazotrophic abundance and community structure in acidic soils in southern China. Soil Biology and Biochemistry, 113: 240–249.

Zhou T.Y., Jiao K.B., Qin S.J., Lyu D.G. (2019): The impact of cover crop shoot decomposition on soil microorganisms in an apple orchard in northeast China. Saudi Journal of Biological Sciences, 26: 1936–1942.

Zou Q. (2006): Experimental Guidance of Plant Physiology. Beijing, China Agricultural Press.

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