

# Composition Response of *Actinomycetes* & *Bacteroidetes* to Different Nitrogen Forms Addition in Rhizosphere Soil of Alfalfa

Yi Zhao<sup>1</sup>, Shengnan Sun<sup>1\*</sup>, Yuqiang Wang<sup>1</sup>, Wentao Liu<sup>1</sup>, Ling Zhu<sup>1</sup>, Luyun Chen<sup>1</sup>, Xuebing Yan<sup>1</sup>

Reasonable nitrogen application dramatically promote the growth of alfalfa and increase its yield by affecting the structure of soil microbial community, but how the addition of nitrogen in different forms and proportions influence the growth of alfalfa is still unclear. Here, a pot experiment with multi-form and multi-proportion nitrogen addition ( $\text{NaNO}_3$ ,  $\text{NH}_4\text{Cl}$ ,  $\text{NaNO}_3:\text{NH}_4\text{Cl}=7:3$ ,  $\text{NaNO}_3:\text{NH}_4\text{Cl}=1:1$ ,  $\text{NaNO}_3:\text{NH}_4\text{Cl}=3:7$ ) was set up in the greenhouse. Nutrient solution without nitrogen was applied at the three-leaf stage of alfalfa, once a week (200mL each time) for six weeks and complete plants of alfalfa were taken at the budding stage. Then, the soil attached to the root system was gently shaken off for subsequent analysis. The results showed that soil total nitrogen (TN), nitrate nitrogen ( $\text{NO}_3\text{-N}$ ) and ammonium nitrogen ( $\text{NH}_4\text{-N}$ ) were significantly increased by different forms and proportions nitrogen addition ( $P<0.05$ ). Nitrogen addition had a positive stimulation on the activity of soil nitrate reductase (NR) and nitrite reductase (NiR). For nitrate reductase (NR), activity in the mixed-nitrogen groups were significantly higher than that of single-nitrogen groups ( $P<0.05$ ). Based on high-throughput sequencing technology, we found that nitrogen form and proportion had no significant effects on the  $\alpha$ -diversity of bacteria in rhizosphere soil ( $P>0.05$ ), but altered their community composition. While soil total nitrogen (TN), ammonium nitrogen ( $\text{NH}_4\text{-N}$ ), nitrate reductase (NR) and nitrite reductase (NiR) were significantly related to both community composition of *Actinobacteria* and *Bacteroidetes*, root biomass of alfalfa only significantly correlated with community composition of *Bacteroidetes* ( $P<0.05$ ). Moreover, mixed nitrogen application significantly increased relative abundance of *Actinobacteria* while decreased relative abundance of *Bacteroidetes* ( $P<0.05$ ). The relative abundance of *Actinobacteria* was significantly positively correlated with NR and NiR, but *Bacteroidetes* was the opposite ( $P<0.05$ ). We also found that nitrogen application significantly ( $P<0.05$ ) improved the aboveground biomass of alfalfa through its effect on soil microbial community, while belowground biomass was increased only with a higher proportion of ammonium nitrogen. These results highlight that *Actinobacteria* and *Bacteroidetes* seemed to respond more sensitively to different proportions of nitrogen and might have prominent roles in soil nitrogen transformation processes.

---

<sup>1</sup>College of Animal Science and Technology, Yangzhou University, Yangzhou 225009, Jiangsu, China

\*Corresponding author: [snsun@yzu.edu.cn](mailto:snsun@yzu.edu.cn)