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# Carbon substrates exert a stronger role than mineral nitrogen application in structuring soil diazotroph communities during Chinese milk vetch growth

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ABSTRACT

Carbon (C) quality and quantity and nitrogen (N) availability are known to play a crucial role in influencing diazotroph community structure in soils and they are commonly affected by crop residue management and fertilizer application. However, a full understanding of how C and N interactions contribute to shaping soil diazotroph communities remains elusive. An experiment comparing two different C substrates (rice straw vs. glucose) plus low to high rates of mineral N application was conducted in two paddy soils with contrasting pH (pH = 6.05 and 7.85) with Chinese milk vetch (CMV) growth. Soils were sampled during the full blooming stage of CMV and diazotroph community structure was characterized using the *nifH* marker gene. The results showed that the diazotroph community responded differently to C substrates (straw and glucose) depending upon the C availability. In both soils, mineral N addition decreased *nifH* gene copy numbers in the straw-included treatments, but not in the glucose-included soils. Compared to the straw-included soils, glucose addition resulted in less α-diversity of diazotroph. Meanwhile, diazotroph community structure was clustered into different groups by the C sources, and marginally affected by N levels. These results suggested that the responses of the diazotroph community to N supply were regulated by C availability. Glucose addition decreased the relative abundance of *Bradyrhizobium* compared to straw incorporation (16.0%–25.9% vs. 19.5%–38.2%), but significantly increased the abundance of the second most dominant genus *Geobacter* (12.8%–23.7% vs. 4.1%–8.7%). In addition, the diazotroph diversity and community structure were less responsive to straw, glucose and mineral N addition in the lower vs. high pH soils. Overall, the results suggest that the responses of diazotrophs to N availability rely on C availability in paddy soils, and that C substrates exert a stronger influence than mineral N application in structuring diazotroph communities.

## 1. Introduction

Biological nitrogen (N) fixation is one of the most import transformations of global N cycle, which contributes 40–387 Tg N $yr^{-1}$ to terrestrial ecosystems (Hsu and Buckley, 2009; Vitousek et al., 2013; Fowler et al., 2015). Diazotrophs are the microbial group involved in this natural process (Levy-Booth et al., 2014). The diazotroph abundance and community structure in soils are frequently shifted by environmental conditions, which can alter their rate of N fixation and have functional ecological significance (DeLuca et al., 2007; Hsu and Buckley, 2009; Smercina et al., 2019). Many studies have revealed that diazotrophs respond substantially to soil physical and chemical changes, including the presence of carbon (C) substrates, nutrient availability, soil pH, oxygen partial pressure, etc. (Collavino et al., 2014; Lin et al., 2018). Farming practices such as organic amendments and fertilizer application commonly alter soil C and N status, and thus are potentially crucial factors influencing diazotroph abundance and community structure.

Exogenous C inputs can provide an abundant source of energy to soil microbes and stimulate diazotroph development, since diazotrophs often metabolize organic matter both to fix $N_2$ and to maintain high respiration rates to avoid $O_2$ inactivation of nitrogenase (Collavino

*Abbreviations:* C, carbon; CMV, Chinese milk vetch; Glc, glucose; N, nitrogen; Rs, rice straw; SOC, soil organic carbon; TN, total nitrogen

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