



## Maintenance of N cycling gene communities with crop-livestock integration management in tropical agriculture systems



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### ABSTRACT

Five different agricultural management systems and a forest fragment at two farms were investigated for effects on soil bacterial indicators. This study was conducted in southern Mato Grosso do Sul, Brazil, in January 2014, at two farms that incorporated integrated crop-livestock systems in Hapludox soil. Impacts of management were assessed through evaluation of functional bacterial communities using quantitative PCR (qPCR) of nitrogen cycling genes (*amoA*, *nirK*, and *nosZ*), PCR-DGGE of the *nirK* community composition, and by investigating relationships of the *nirK* community with soil properties. The *nirK* gene community showed that reference forest soil maintained relatively large *nirK*, *nosZ*, but small *amoA* gene abundances and large range weighted *nirK* richness (*Rr*). The introduction of pasture into crop-livestock integration management (inclusion of the second and third year of grazing within a crop-livestock integration system, CL-2 and CL-3, respectively) or the field in its first year of crop rotation after grazing pasture (CL-b) increased *nosZ* and *amoA* abundance. Three years of grazing pasture in crop-livestock integration (CL-a) increased *nirK* abundance, while multiple years of crop rotation in crop-livestock integration management systems increased soil OM and promoted a richer *nirK* community composition. The *nirK* community *Rr* that was not significantly different from the forest was observed in the second or third year of crop rotation after grazing (CL-c or CL-d, respectively) and (CL-3). In contrast, fields in pasture (CL-a, continuous pasture (CP)) and the first year of crop rotation after pasture (CL-b) had reduced *Rr* and functional organization curves, indicating communities less resilient to future stress. Management systems under integrated crop-livestock farming in tropical soil maintained a relatively diverse N cycling bacterial community, possibly promoting soil quality and N cycling processes.

### 1. Introduction

The ultimate challenge for agricultural production is to achieve the highest level of productivity within the context of natural resources (e.g. soil and water) conservation. Integrated row crop and livestock production systems are promoted as a means to help meet those simultaneous goals of maximizing productivity and soil conservation in Brazilian and other temperate and tropical agriculture systems (Palm et al., 2014; Lemaire et al., 2014). A major concern in Brazil is changing land use from native, often forested, vegetation to agriculture which can result in land degradation (Mendes et al., 2015). Consequences of land degradation include loss of productivity of the land, decreased resilience to perturbations, increased economic costs, and increased

contribution of greenhouse gas emissions (Bento et al., 2018). Soil management is a critical element of sustainable agricultural production because agricultural management practices can modify soil properties in magnitude and direction, which may either improve or reduce soil quality (Bono et al., 2013). The maintenance or improvement of soil quality can be related to greater soil organic matter content, moisture, and nutrient retention (Kibblewhite et al., 2008; Bhardwaj et al., 2011).

Approaches to farming that integrate productivity with soil and water conservation have led away from simplified systems to more diversified agricultural and livestock management systems, such as crop-livestock integration (CL) with soybean or corn along with *Brachiaria* pastures in Brazil (Machado et al., 2011). In soil classified as very clayey texture Oxisol, crop rotation in a crop-livestock integrated

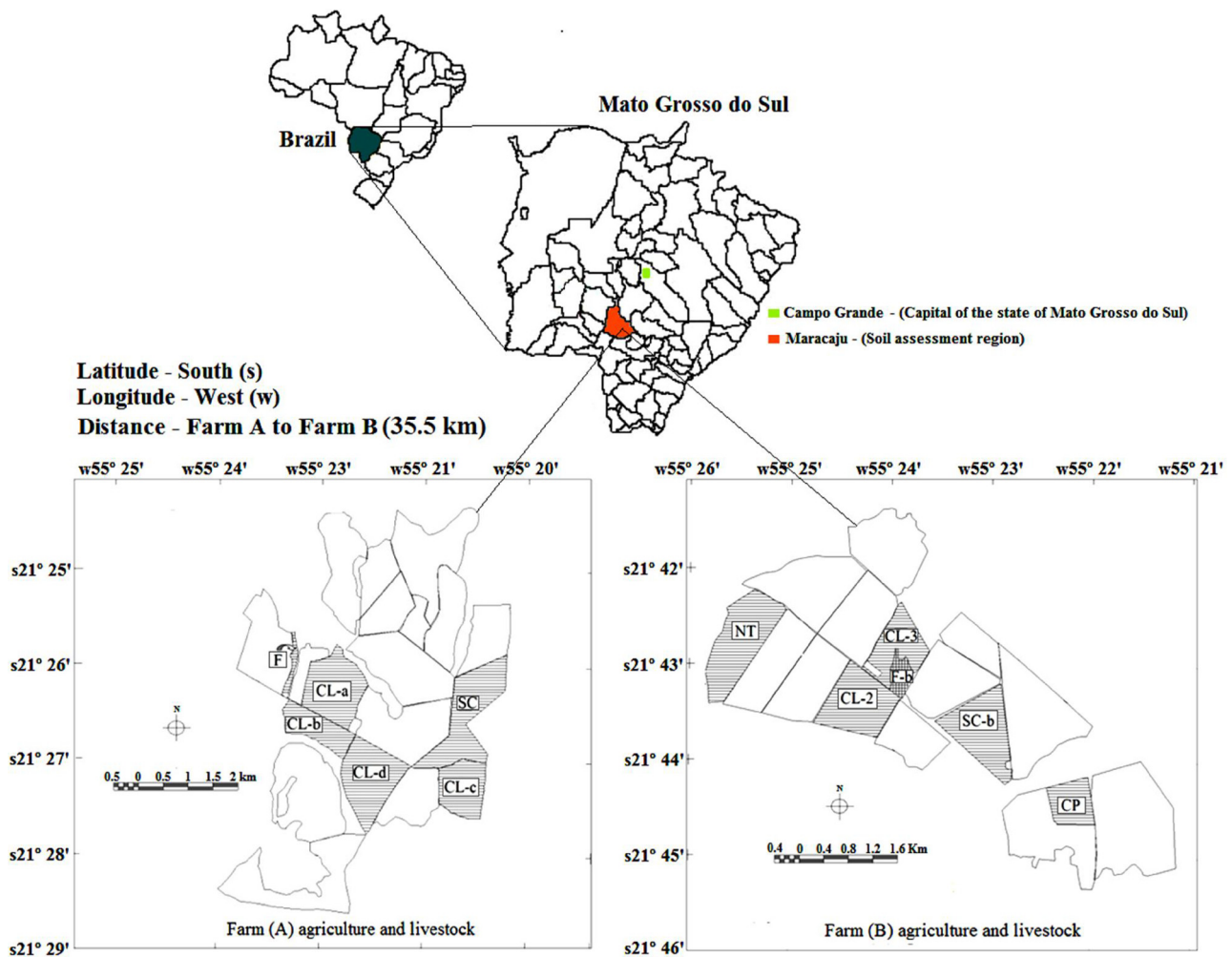
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**Fig. 1.** Maps of the agricultural management systems at two farms (A) and (B) in the region of Maracaju, Mato Grosso do Sul, Brazil. Farm A: CL-a, crop-livestock integration system: third year pasture; CL-b, first year of crop rotation after grazing; CL-c, second year of crop rotation after grazing; CL-d, third year of crop rotation after grazing; SC, sugarcane with mechanized harvesting. F, forest fragment. Farm B: CL-2, second year of crop-livestock integration cycle; CL-3, third year of crop-livestock integration cycle; NT, no-tillage; SC-b, sugarcane with mechanized harvesting; CP, continuous pasture; F-b, forest fragment.

system enabled the maintenance of soil quality and soil carbon levels equal to the native forest, and provided greater capability of the system to withstand disturbances in the 0–5 cm soil layer (Tirloni et al., 2012). However, how biological communities respond to these integrated management systems remains unclear (Caetano et al., 2013; Pezarico et al., 2013).

Soil microorganisms have shown great sensitivity to changes in agro-ecosystems (Rosa et al., 2014) and can thus serve as biological indicators, allowing researchers to infer environmental quality or the effects of an agent, process, or integration of decisions on parameters in the environment (Casalinho et al., 2007). The use of molecular biology techniques allows analysis of soil microbial communities without the reliance and limitations of growing microorganisms in culture (Marzorati et al., 2008). Molecular markers have been used to detect changes in bacterial communities impacted by agricultural practices in Brazil (Peixoto et al., 2006). However, there is still much that is not understood about how agricultural practices alter bacterial communities, especially functional bacteria involved in the cycling of nitrogen. As such, the bacterial *amoA* gene is used as a marker for bacterial nitrification, while *nirK* and *nosZ* genes are often targeted to assess denitrifier abundance (Philippot et al., 2011). The *nosZ* gene in particular serves as a marker for nitrous oxide reduction (Butterbach-Bahl et al., 2013).

Studies of specific denitrifier genes in tropical agricultural

production systems are limited (Henry et al., 2008; Morales et al., 2010). Denitrifiers comprise about 5% of the soil bacterial community and include a diverse subset of facultative anaerobic bacteria participating in the reduction pathway of nitrate ( $\text{NO}_3^-$ ) to nitrite ( $\text{NO}_2^-$ ), nitric oxide, nitrous oxide, and eventually to molecular nitrogen ( $\text{N}_2$ ) (Koch et al., 2015). Given the importance of nitrogen to plant growth and development and the potential of nitrogen loss through nitrification followed by denitrification contributing to nitrogenous greenhouse gas emissions from terrestrial systems, better understanding is needed of how production systems impact organisms involved in nitrogen cycling (Philippot and Hallin, 2006). A wide distribution of taxonomically diverse organisms carry the *nirK* gene, contributing to the reduction of nitrate to nitrite in soil (Throback et al., 2004; Enwall et al., 2010; Orlando et al., 2012). Given the potential of production systems to impact so many variables in soil that influence denitrification, e.g. organic matter, pH, nutrients, moisture, and soil aggregate structure, it needs to be understood how crop-livestock integration systems in Brazilian agriculture change denitrifier communities in relationship to chemical and physical soil properties (Meyer et al., 2013; Moraes et al., 2014).

Here we propose that functional bacterial communities involved in nitrogen cycling processes can be used as biological indicators to monitor the soil environment in agriculture (Peixoto et al., 2010). Therefore, to evaluate nitrifying and denitrifying microorganisms in

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