Rhizobium-white clover symbiosis and nitrogen fixation along a soil organic carbon gradient

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Introduction

Sustainable cultivated pasture production in the southern Cape region of South Africa requires management practices that will support soil quality (Swanepoel and Botha 2012). Soil organic carbon (C_{org}) sequestration is regarded as one of the most important ways to promote soil quality. Dairy farmers in the southern Cape attempt to increase C_{org} by following minimum-tillage practices. These irrigated dairy-pastures require high levels of nitrogen (N) to maintain highly productive pastures. Nitrogen fertilisers have become very expensive and severely strain profitability of these dairy-pastures. Forage legumes are frequently incorporated into the grass pastures to overcome economic and environmental problems associated with high levels of inorganic fertiliser application. Their capacity to fixate atmospheric N contributes greatly to nutritional value, palatability and subsequently profitability of pastures (Botha 2003). Since C and N are biophilic compounds, C_{org} may have a direct effect on the N-fixating legume plant or the *Rhizobium* population that infects its roots (Swanepoel *et al*. 2011).

The aim of this study was to determine the effects of C_{org} on white clover (*Trifolium repens*) biomass production and N-fixation by host-specific *Rhizobium*.

Materials and Methods

This study was carried out on Outeniqua Research Farm (South Africa). Soils of similar textures, but with different levels of C_{org} were used in a pot trial with white clover cv. Haifa. A standard soil analysis showed that the nutrient content were within recommended soil fertility levels for white clover. The experiment was a factorial design with nine replications: two levels of inoculation (seeds inoculated with *Rhizobium leguminosarum* bv. *trifolii*, and seeds not inoculated) at five levels of C_{org}. Cape weed (*Arctotheca calendula*) was sown in nine pots which were randomly distributed amongst the other pots as non N-fixing reference plants used to quantify N fixation (Carranca *et al*. 1999). Enumeration of symbiotic *Rhizobium* capable of infecting white clover was determined by plant infection technique (Woomer *et al*. 1990). Symbiotic effectiveness was measured as biomass weight. C_{org} was determined by the Walkley-Black method (Walkley 1935) and total N by the AgriLASA method (AOAC International 2002). An analysis of variance with linear contrasts and log transformations was performed for the continuous variables. The data was acceptably normally distributed. A student t-test at a 5% significance level was used to determine least significant differences between means.

Results and Discussion

Rhizobium was detected in all soils, regardless of level of C_{org} or inoculation, stressing the robustness and adaptability of these important bacteria in agricultural soil. The MPN of symbiotic *Rhizobium* cells ranged from 78 to 8900 cells/g soil and was affected by C_{org} levels, but not consistently (Fig. 1). Inoculation had no effect (P>0.05) on the most probable number of symbiotic *Rhizobium* cells in soil within a C_{org} level. Total N had a very strong positive correlation with C_{org} (r^2 = 0.99). The amount of N fixed by *Rhizobium*-associated root nodules was very strong, but negatively correlated to C_{org} (r^2 = -0.90), which could be expected, since white clover was more reliant on atmospheric N in low C soils. Linear regressions for prediction of biomass production from C_{org} are shown in Figure 2. It is clear that enhanced C_{org} levels have led to higher biomass production. Although less N was fixed by *Rhizobium*-

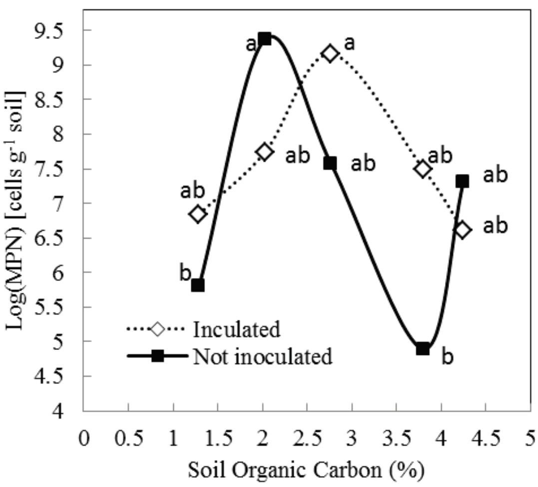

Figure 1. Most-Probable-Number (MPN) values (log transformed) of symbiotic Rhizobium bacteria at different soil organic carbon levels, planted with inoculated or non-inoculated white clover seed. Least significant difference (*P*=0.05) = 2.86; MPN values with no common letter differed significantly (*P*=0.05).

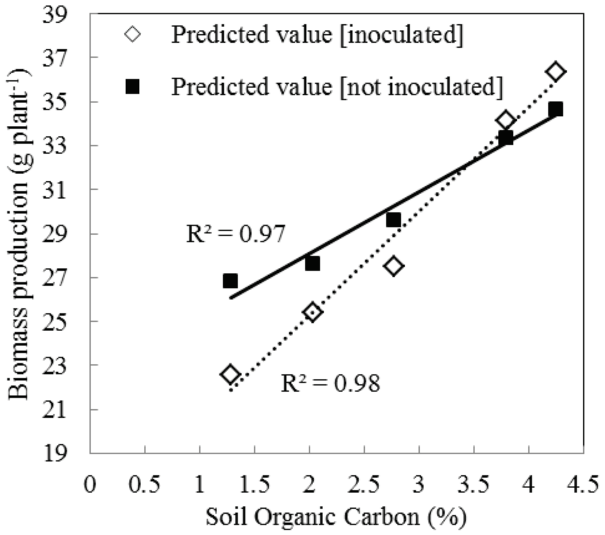

Figure 2. Mean biomass production (dry weight) of white clover roots and shoots as affected by soil organic carbon content where seeds were either inoculated with *Rhizobium leguminosarum* bv. *trifolii* or not inoculated. Linear regressions were highly significant (*P*<0.001).

associated nodules in roots of white clover, biomass production remained higher in soil with high C_{org} levels. This may be a combined result of increased C_{org} levels and secondary soil characteristics associated with enhanced levels of soil organic matter, such as lower bulk density, higher aggregate stability and increased water holding capacity.

Conclusion

Rhizobium leguminosarum bv. *trifolli*, being an indicator of soil health, is a common and beneficial bacterial species in pasture soils in the southern Cape region of South Africa. Management strategies to increase C_{org} content of pastures containing white clover are necessary to maximise the efficiency of N fixation and biomass production. Researchers in the southern Cape need to give attention to soil quality to support sustainability of pasture systems.

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