



Examining trophic-level nematode community structure and nitrogen mineralization to assess local effective microorganisms' role in nitrogen availability of swine effluent to forage crops

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ABSTRACT

Culturing and using effective microorganisms from locally derived microorganisms has become a common practice employed by farmers throughout Central America as well as parts of South America and Southeast Asia to control foul odors and flies around compost piles and livestock facilities. These local, effective microorganisms (LEM) are applied in a concentrated solution fermented from carbohydrate-rich substrates inoculated with locally-sourced, actively decomposing leaf litter. Despite its growing prevalence among small-scale farmers in some parts of the world, there is little published research that explores the beneficial or detrimental effects of changing the biome of animal-based nutrients and the soils to which LEM are applied. The objectives of this study were to evaluate the effect of combining LEM with swine effluent to fertilize annual ryegrass (*Lolium multiflorum*) on plant-available nitrogen, nematode community structure, and forage productivity. Forage performance was good across all of the treatments – with relative feed quality (RFQ) values above 200 and 100 in the March and May harvests respectively. In the first, second and third sampling dates, soils from 0 to 5 cm depth showed more nitrogen mineralized in LEM-treated soils than in control soils ($p = 0.178$, $p < 0.0001$ and $p = 0.027$) respectively. After nutrient applications, soil-nematode community structure (0–10 cm) at the trophic group level changed over time. Most changes were similar between treatments, however, plant parasitic nematode populations were found to be significantly higher in LEM-treated plots in all but one of the sampling dates. LEM is gaining popularity as a natural amendment and although we have shown that it does increase N availability and changes trophic-level nematode community structure, more research is needed to optimize the potential of these benefits.

1. Introduction

Animal manures and organic wastes serve as important nutrient resources for small and large-scale farmers around the world. However, the availability of the nitrogen present in these materials may be low, as it is released slowly during the decomposition of these amendments. The plant-available nitrogen coefficients for organic amendments can range from 0.05 to 0.8 (Baldwin and Greenfield, 2006) and are dependent upon a number of factors, including environmental and soil conditions, C:N ratio of the amendments, and on the biome of decomposers: N mineralizers and scavengers in the soil.

Several products and approaches have been attempted to fortify the soil biome. Research in the early 1970s at the University of the Ryukyus, Okinawa, Japan resulted in the development of effective

microorganisms (EMTM), which was a mixed culture of beneficial and naturally occurring microorganisms, including species of photosynthetic bacteria, lactobacilli, yeasts, and actinomycetes (Higa, 2000). The purpose of these beneficial organisms is to improve crop growth, yield, and/or quality by increasing soil nutrient availability; producing bioactive substances such as hormones, enzymes, and growth promoters; controlling soil-borne pathogens; and accelerating decomposition of lignified materials in the soil (EM Research Organization, 2016). Though EMTM was produced and distributed by EM Research Organization, Inc. (Uruma City, Okinawa, Japan) and its licensees, locally-produced versions of the commercial microbial inoculant have been cultured and applied in many regions. For example, farmers in Central and South America, produce and use a microbial product called microorganismos de la montaña (mountain microorganisms) that is

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brewed from carbohydrate-rich substrates inoculated with locally-sourced microbes found in partially decomposed leaf litter. These local, effective microorganisms (LEM) have gained popularity because of a demonstrated ability to reduce foul odors and flies when applied to composts, dairy corrals, pig pens, and chicken coops. Agricultural extension organizations throughout Central and South America, such as the Advisory Foundation for the Ciudad de Dios Rural Sector (as La Fundación de Asesorías para el Sector Rural Ciudad de Dios; FUNDASES) in Colombia, The Ministry of Agriculture and Livestock (el Ministerio de Agricultura y Ganadería; MAG) in Costa Rica (Tencio, 2016), and The National Association for the Promotion of Agroecology (la Asociación Nacional para el Fomento de la Agricultura Ecológica; ANAFAE) in Honduras (ANAFAE, 2016), have actively promoted the use of LEM. Despite its widespread use, there is little published research that explores the potential benefits or challenges associated with changing soil ecology when different nutrient sources are applied.

Though there has been some research conducted concerning the ability of commercial EMTM inoculant to improve soil function and crop productivity, reports of its effectiveness vary. While several researchers found positive effects of EMTM additions to fertilizer regimes on crop yield and/or soil fertility (Daly and Stewart, 1999; Daur and Abusuwar, 2015; Javaid and Bajwa, 2010), others found little or no beneficial effects from incorporating EMTM into production (Mayer et al., 2010; Zu Schwienberg-Mickan and Müller, 2009; Vliet et al., 2006), there has been little work done investigating the effectiveness or consequences of the locally-produced LEM inoculant on soil function and crop productivity. The research that has been published suggests that it has the potential to improve agricultural production. For example, Kamla et al. (2008) investigated the effects of a locally-produced microbial inoculant similar to LEM on cowpea (*Vigna unguiculata* (L.) Walp.) yield and found that when used in combination with an organic amendment, the fermented bio-extract (LEM) resulted in higher yields than those achieved with the addition of the organic amendment alone. It is possible that the use of locally derived microbes to make LEM is not only more affordable and accessible to farmers, but it may also be more effective than the commercial equivalent. Campo-Martinez et al. (2014) investigated the effects of both EMTM and locally-produced, LEM/MM bacterial inoculants on the growth of chard and found that the LEM performed significantly better than the commercial EMTM product.

As is evidenced by some of the contrasting results of previous research, the efficacy of LEM is promising but may be affected by many factors including climate, soil type, organic material, crop type, etc. Indirect effects of microbial changes such as nitrogen mineralization are one way to examine changes that may be taking place among microbial populations in a soil ecosystem. Nitrogen mineralization is an important, biologically-driven soil function through which nitrogen compounds contained in organic materials such as vegetative matter, manures, and dead organisms, are mineralized into inorganic N (NH_4^+ and NO_3^-) and available for plant uptake. Studying the mineralization of organic N in a soil can provide information about the relative size and activity of the soil microbe community (Bengtsson et al., 2003; Woods et al., 1982) and also provides a measure of soil fertility (Crohn, 2004).

Organisms such as free-living nematodes, which occupy key positions as primary and intermediate consumers in the soil food web, can also be used as measures of soil properties related with their nutrient resources. Ecologists have recognized the usefulness of nematodes as proxies for environmental and soil health since the 1970s (Ferris et al., 2012). Practical analysis of changes in nematode population structure is possible due to their abundance in soil ecosystems, the ease with which they can be extracted, and the ability to easily identify individuals to trophic groups or family using esophageal morphology (Fu et al., 2000; Nair and Ngouajio, 2012; Parmelee et al., 1995). For this research, we categorized nematodes into bacterial-feeding nematodes, fungal-feeding nematodes, plant parasitic nematodes (PPN), Tylenchidae, Dorylaimidae, and Mononchidae. The last three categories are families

of nematodes most often associated with fungal or epidermal root-feeding, omnivorous feeding and predatory feeding, respectively. Being one or two steps higher in the food chain than bacteria and fungi, nematodes serve as integrators of physical, chemical, and biological properties related with their food resources (Neher, 2001). Microbial grazing nematodes, for instance, can regulate rates of decomposition and nutrient availability in the soil by affecting growth and metabolic activity of microbial communities.

We hypothesized, if LEM is incorporated into organic amendments such as swine effluent and applied to soils, nematode assemblage structure will shift towards being more dominant in bacterial and fungal grazers, resulting in an increase in nitrogen mineralization and in an increase in plant productivity. Based on this hypothesis our objectives were to measure and determine differences between treatments of either swine effluent (Control), swine effluent with False LEM (FLEM) or swine effluent with LEM in 1) nematode abundance and trophic group structure, 2) differences in soils' potential to mineralize nitrogen, and 3) plant productivity and quality.

2. Materials and methods

2.1. Site description and design

This study was conducted in the southeastern United States at the J. Phil Campbell Research and Education Center in Watkinsville, Georgia, (33°52' N, 83°27' W). The soil at the site is a fine kaolinitic, thermic Typic Kanhapludults. It is in the Cecil sandy loam series with a 2–6% slope (Soil Survey Staff, 2006.). The region has 123 cm average annual rainfall and an average minimum and maximum temperature of 10.4 °C and 22.5 °C. The soil temperature in the plots ranged from 9 °C to 16 °C during the course of our research.

Each of the treatments were applied with swine effluent. The treatments compared were local effective microorganisms (LEM), made by cultivating the O horizon biome of the forest floor with a growing media under anaerobic conditions; False-LEM (FLEM), made with the same growing media as LEM but without the O-horizon, under the same conditions; and a ground water control. After six weeks, the solid LEM and FLEM were extracted in a 1:16 sugar:water solution. Additional details on producing the LEM and FLEM inoculants can be found in [Supplementary Materials](#).

Plots were arranged in a randomized complete block design with four replications of each treatment. Individual plot size was 18 m² (3 m × 6 m). The experiment was carried out for two growing seasons, 2014–15 and 2015–16, beginning in November and ending in March. Plots were established in annual ryegrass (*Lolium multiflorum* L.) each November. After tilling and cultipacking, ryegrass was planted with a Hege 80 plot drill at a depth of ½ inch, at a rate of 25 lb/acre. Three different treatments – LEM, FLEM and Control – were combined with an organic nutrient source and surface-applied to the forages. The amendment used was liquid swine effluent collected from the UGA swine research facility.

Nutrient analysis of the effluent was made at collection, just prior to application. Applications of 11.0 L ha⁻¹ were made on 4 December 2014 and 6 April 2015 in the 2014–15 season, using effluent containing concentrations of total nitrogen 2115 and 3048 ppm, respectively. Applications of 5.5 L ha⁻¹ were made on 11 November 2015 and 18 February 2016 in the 2015–16 season, using effluent containing concentrations of total nitrogen 2355 and 1532 ppm, respectively. Therefore, the total N applied was 50 kg N ha⁻¹ in the 2014–15 season and 27 kg N ha⁻¹ in the 2015–16 season.

2.2. LEM/FLEM microbial community

Samples of the liquid LEM and FLEM were taken before the first application each year for DNA analysis. Liquid FLEM and LEM samples were extracted with Qiagen DNA extraction kit. Metagenomics analysis

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