

INFLUENCE OF JAPANESE BEETLE LARVAE ON SOIL MICROBIAL DIVERSITY AND ACTIVITY

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Introduction

The Japanese beetle *Popillia japonica* Newman (Coleoptera: Scarbeidae) (JB) is an invasive pest species that does serious damage to a wide range of agricultural and horticultural crops including turfgrass. The adult, which feeds on over 400 species of plants (2002) (Potter & Held), is easy to identify with its metallic green head and coppery elytra. The larvae are small white C-shaped grubs, less than an inch long with 3 pairs of easily visible legs. While much is known about the biology of the adult, details about the ecological interactions between the larva and its underground environment have received much less attention. Understanding how these insects influence soil microbial communities could provide an important portal into the nature of these interactions that could have implications for global challenges ranging from invasive species to climate change. In working toward this goal, the objective of this study was to examine how soil dwelling JB larvae influence soil microbial diversity and respiration. Using a series of short-term laboratory microcosm experiments, this study tested the hypothesis that JB larval infestations alter soil phospholipid fatty acid profiles (microbial diversity) and respiration patterns (microbial activity) thereby leaving a biological footprint in the soil.

Materials and Methods

Japanese beetle larvae were collected from the field during October 2012. At that same time, soil was collected from un-infested plots to a depth of 7.5 centimeters and placed into plastic totes. All white grubs were kept in the soil collected from infested plots and all soil and white grubs were returned to the laboratory where they were stored in an incubator at 10°C until needed.

Soil Microbial Assessment. Nine glass canning jars (0.47 L) were arranged on the laboratory bench and 60 g of uninfested soil was added to each. In three replicate jars, 0, 5 or 10 white grubs were added, respectively. Canning lids were modified by drilling one hole (6.35 mm) through the lid and forcing a rubber septum into the hole to again make the lid air-tight. These lids were placed on all jars and the jars were incubated on the bench at room temperature (21°C) for 12 hours. After this time, larvae were removed from the jars and the soil from each jar was placed into glass vials. Vials were held at -80°C until processed for phospholipid fatty acid analysis (PLFA) following the methods of Acosta-Martinez et al. (1999) (Ward Labs, Kearney, NE) which was used to quantify microbial biomass and characterize the structure of the microbial community. Although each treatment was replicated three times, soil from each respective treatment was pooled prior to PLFA analysis.

Soil Microbial Respiration: Nine glass canning jars (0.47 L) were arranged on the laboratory bench and 60 g of uninfested soil was added to each. In three replicate jars, 0, 5 or 10 white grubs were added, respectively. Canning lids were modified by drilling one hole (6.35 mm) through the lid and forcing a rubber septum into the hole to again make the lid air-tight. These lids were placed on all jars and the jars were incubated on the bench at room temperature (21°C) for 12 hours. After this time, air samples were taken (12cc) through the septum. These samples were transferred using the technique described in Koerner et al 2011 to the autosampler vial (2cc) and then tested with the gas chromatography-mass spectrometer (GC-MS). All larvae were removed from the jars and the jars were resealed and left to sit on the bench at room temperature for an additional 12 hours. After this time, levels of CO₂ within the head-space of each jar were quantified using the same methods as above.

Results

PLFA analysis indicated that soil microbial diversity decreased as JB larval increased (Figure 1) with populations of rhizobia, fungi and protozoa decreasing as an overall proportion of the microbial community while populations of bacteria increased.

Headspace CO₂ concentrations increased linearly with increased JB larval density 12 hours after the infestation phase of the experiment (F=5.4; df=1, 10; P=0.04) (Figure 2). Twelve hours after JB larvae were removed from the jars, there was still a tendency for headspace CO₂ concentrations to increase with JB density, but this increase was not statistically significant (F=2.7; df=1, 10; P=0.13) (Figure3).

Conclusions

The JB larvae ingest and process large amounts of soil and the data collected during this experiment indicates that these larvae significantly reduce soil microbial diversity, specifically rhizobial populations. This could seriously alter the underground environment and change soil function. Rhizobia are free living soil bacteria that play a major role in nitrogen fixation. Once atmospheric nitrogen is fixed and the N-N bond is broken, the atoms are free to make different bonds and form other chemical compounds necessary for plant survival. For some plant species, rhizobia must be living in plant tissue for them to effectively fix atmospheric nitrogen. Since nitrogen is essential for plant growth and development, the relationship that rhizobia have with plants is mutualistic (1990) (Lindemann & Glover). The ability of JB larvae to reduce rhizobial populations could have implications for future plant growth following infestation. Further research efforts should examine the time required for rhizobia to stabilize once JB larvae have been eradicated, and determine if JB infestations do in fact influence future plant growth.

The hindgut of the soil-dwelling scarab larvae is modified into a microbe rich fermentation chamber that facilitates the acquisition of nutrients from the relatively nutrient poor soil matrix. Not

surprisingly, the results of this study indicate that JB infestations cause a shift in the soil microbial community in favor of bacteria, though identity of these bacteria was not determined. The data indicate the bacteria may increase the rate at which carbon is liberated from the soil as indicated by the continued respiration after removal of the grubs. Soil is the largest carbon pool on earth and, according to the USEPA, carbon dioxide comprises 82% of the greenhouse gases emitted by the United States (USEPA, 2015). Because of the density of larvae present in heavily infested soil (upwards of 500 larvae/m² have been observed) their ability to erode the carbon storage capacity of the soil should be further examined. It may be possible to quantify the impact of JB infestation on carbon sequestration on a per unit area basis thereby providing a measure of the impact this invasive species has on important ecosystem services related to climate change.

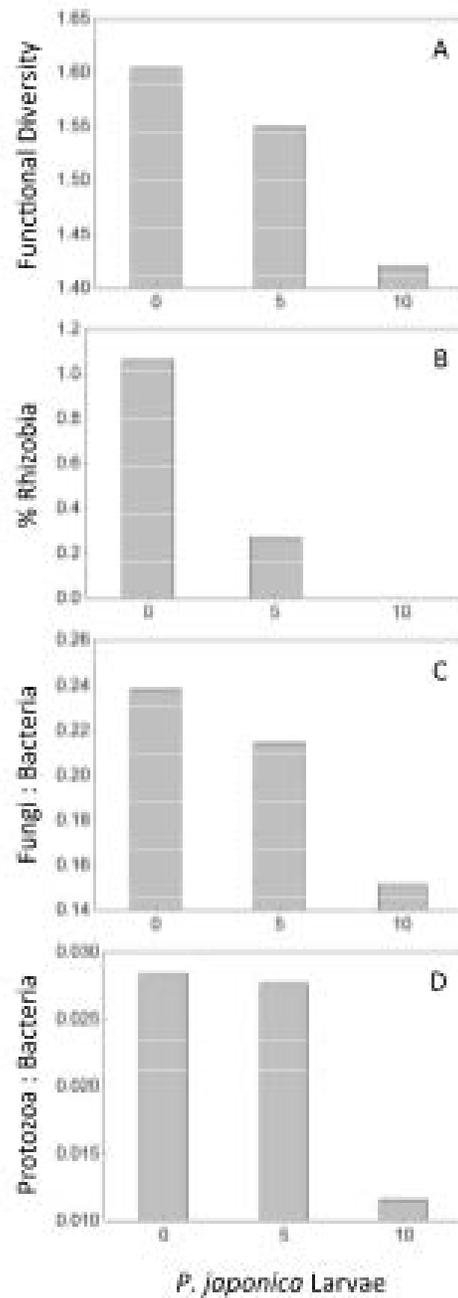


Figure 1. Soil microbial diversity (A), total biomass of rhizobia (B), fungi-bacteria ratio (C), and protozoa-bacteria ratio (C) of 60g soil samples incubated for 12 hours in glass jars containing previously infested soil with no larvae, and clean field soil infested with 5 or 10 3rd instar Japanese beetle larvae.

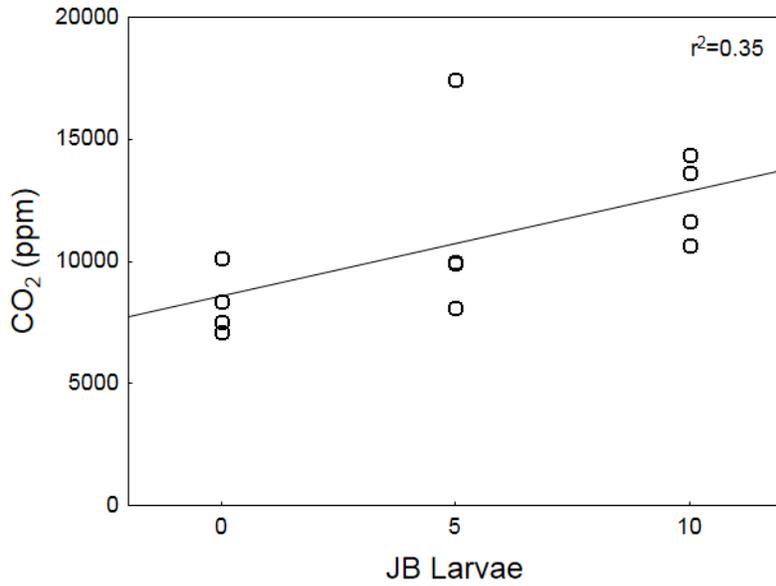


Figure 2. Headspace CO₂ concentrations (ppm) in glass jars containing 60 g of previously uninfested field soil and 0, 5 or 10 3rd instar Japanese beetle larvae after 12 hours of incubation in the laboratory at 21°C.

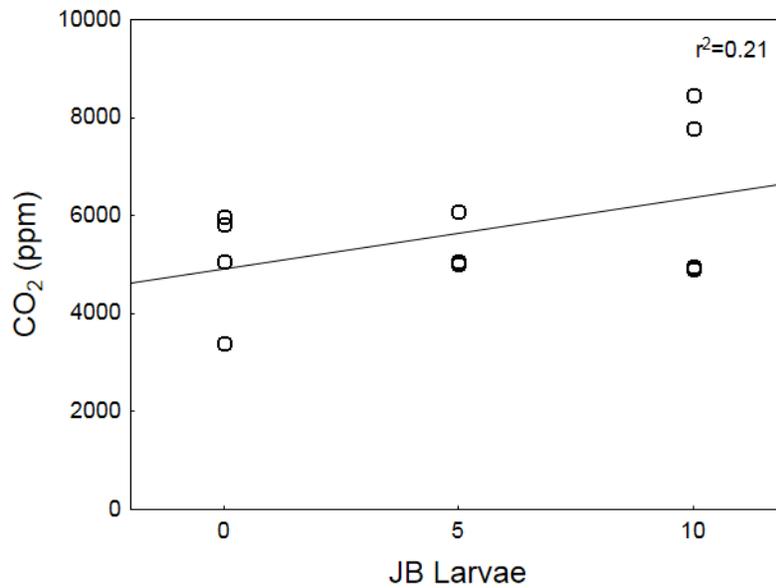


Figure 3. Headspace CO₂ concentrations (ppm) in glass jars containing 60 g of soil previously infested by 0, 5 or 10 3rd instar Japanese beetle larvae for 12 hours. Soil was incubated for 12 hours in the laboratory at 21°C after larvae were removed.

Citations

Acosta-Martínez, V., et al. (1999). The role of tree leaf mulch and nitrogen fertilizer on turfgrass soil quality. *Biol Fertil Soils*. 29:55-61.

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