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Short communication

Soil invertebrates in Australian rain gardens and their potential roles in storage and processing of nitrogen

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ABSTRACT

Research on rain gardens generally focuses on hydrology, geochemistry, and vegetation. The role of soil invertebrates has largely been overlooked, despite their well-known impacts on soil nutrient storage, removal, and processing. Surveys of three rain gardens in Melbourne, Australia, revealed a soil invertebrate community structure that differed significantly among sites but was stable across sampling dates (July 2013 and April 2014). Megadrilacea (earthworms), Enchytraeidae (potworms), and Collembola (springtails) were abundant in all sites, and together accounted for a median of 80% of total soil invertebrate abundance. Earthworms were positively correlated to soil organic matter content, but the abundances of other taxonomic groups were not strongly related to organic matter content, plant cover, or root biomass across sites. While less than 5% of total soil N was estimated to be stored in the body tissues of these three taxa, and estimated N gas emissions from earthworms (N_2O and N_2) were low, ingestion and processing of soil was high (e.g., up to 417% of the upper 5 cm of soil ingested by earthworms annually in one site), suggesting that the contribution of these organisms to N cycling in rain gardens may be substantial. Thus, invertebrate communities represent an overlooked feature of rain garden design that can play an important role in the structure and function of these systems.

1. Introduction

Rain gardens (also known as biofilters and bioretention systems) are small, terrestrial natural treatment systems designed to filter pollutants from stormwater using porous filter media planted with one or more species of vegetation (Ambrose and Winfrey, 2015; Askarizadeh et al., 2015). Soon after rain garden construction, soil invertebrate communities develop (Ayers, 2009) that may influence important rain garden functions such as infiltration and nutrient retention or removal (Levin and Mehring, 2015; Mehring and Levin, 2015). Though it has yet to be tested in the context of rain gardens, substantial amounts of carbon (C), nitrogen (N) and phosphorous (P) may be stored in soil invertebrate biomass (Teuben and Verhoef, 1992), which temporarily immobilizes nutrients and prevents them from being leached. Soil invertebrates may also impact nutrient retention indirectly through increasing nutrient uptake by plants. The effects of earthworm activity on plant uptake of N are particularly dramatic, with some species reported to enhance uptake >200% in vertical-flow wetlands (Xu et al., 2013). Springtails, millipedes (Diplopoda), and isopods also have the potential to enhance plant uptake of N due to high levels of plant-available N in their fecal material (Anderson et al., 1983; Teuben and Roelofsma, 1990).

Soil invertebrates may play an important role in nutrient removal in rain gardens as well as nutrient immobilization/retention. Earthworms, for instance, have the potential to increase nitrate removal via denitrification because anoxic conditions within their guts favor production of dinitrogen (N_2) and nitrous oxide (N_2O) by ingested soil-derived microbes (Horn et al., 2006), even when surrounding soil conditions are aerobic. Indeed earthworm casts themselves may be denitrification hotspots, prolonging the effects of "worm facilitated" N removal long after excretion (Parkin and Berry, 1994).

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Despite their notable effects on soil biogeochemistry, few studies to date have quantified soil invertebrates in rain gardens. Those studies that have, emphasize above-ground invertebrates (Kazemi et al., 2009a, 2009b), leaving within-soil diversity (and biogeochemical roles) in rain gardens largely unknown. Here we present results from one of the first studies of below-ground invertebrate communities in rain gardens. Our study took place in July 2013 and April 2014 in Melbourne, Australia, and was designed to (1) assess spatial and temporal patterns in invertebrate community structure within rain gardens (2) identify drivers of invertebrate abundance within raingardens (e.g., vegetation type, vegetation cover, and soil organic matter content), and (3) determine if soil invertebrates are likely to contribute substantially to nutrient retention/removal within rain gardens, based on the nutrient content in their biomass, soil processing capacity, and estimates of their contribution to denitrification.

2. Methods

2.1. Study sites

Three rain gardens near Melbourne, Victoria (Australia) were sampled in the winter of 2013 (July) and autumn of 2014 (March–April): (1) "Hereford Road" (HR) is a 100-m^2 rain garden that treats runoff from a 0.93-ha peri-urban catchment in the town of Mt. Evelyn; (2) "Wicks Reserve" (WR) is a 1900 m^2 rain garden that treats runoff from a 11.43-ha peri-urban catchment in the town of The Basin; and (3) "Lynbrook Boulevard" (LB) is a bioretention swale that treats runoff from a 2.0-ha suburban development known as Lynbrook Estate. More information on these rain gardens can be found in Supplementary material (Supplementary material, Table S2).

2.2. Field sampling methods

Samples were collected at points evenly spaced along a transect, from the inlet (where stormwater flows into the rain garden) to the outlet (where excess water leaves the rain garden). At least four points were sampled in each site per sampling season, with 9, 10, and 13 points sampled in total at LB, WR, and HR, respectively. At each sampling location, a 0.25 m × 0.25 m quadrat was used to determine the percent cover of three commonly-planted vegetation types: grasses, sedges, and rushes. Filter media samples (top 5 cm of soil media) were collected from within the quadrat using a plexiglass corer 5 cm in diameter. Because we occasionally did not have immediate access to a laboratory, filter media cores were fixed in 10% phosphate-buffered formalin immediately after collection, and shipped to the University of California, San Diego (UCSD) for analysis of invertebrate community structure and filter media organic matter content.

Additional sub-samples of filter media were collected at two depths (<2 cm and ~10 cm) along the transects described above. Within each site, all sub-samples from a given depth were composited with other samples from the same depth, and placed into glass sample jars for future N content analysis. All composites were frozen within 8 h of collection and stored at −20 °C prior to analysis. Due to concerns that formalin fixation could prevent accurate measurement of N content in invertebrates, estimation was chosen in lieu of direct measurements (see Supplementary material, Appendix S2 for calculations).

2.3. Laboratory methods

Formalin-preserved biofilter media (soils) were rinsed over nested sieves to separate invertebrates, organic matter, roots, and inorganic matter into three size fractions: 1) >2 mm, 2) 0.3–2 mm, and 3) 0.045–0.3 mm. Soil size fractions ≥0.3 mm were sorted under a Wild M5A stereomicroscope at 12× magnification in order to remove all invertebrates, which were then classified according to order, suborder, or family, and enumerated. The most abundant invertebrates (Oligochaetes, including Megadrilacea [earthworms] and Enchytraeidae [potworms]; and Collembola [springtails]) were dried and individual weights were measured in order to estimate biomass per site, body tissue N content, and ingestion rates. Tissue N content and ingestion rates by oligochaetes were estimated using conversion factors (see Appendix S2 in Supplementary material). Following removal of invertebrates and roots, each filter media size fraction was dried to a constant weight at 60 °C, combusted at 500 °C, and re-weighed in order to estimate soil organic matter content as ash-free dry mass (AFDM). Frozen composite filter media samples (2 depths) were sent to a NATA accredited laboratory (http://www.nata.asn.au/) for analysis of total N using standard methods and quality assurance procedures (APHA, 2012).

2.4. Statistical analysis

Overall invertebrate community composition was explored using multivariate analyses (MDS, ANOSIM, SIMPER) run using PRIMER 6 (Primer-E 2006, Plymouth Marine Laboratory, Clarke 1993, Clarke and Warwick, 1994) on fourth-root transformed, unstandardized data (data provided in Supplementary material, Table S1). Also using PRIMER 6, patterns of species diversity among rain gardens were compared by using a sample-based rarefaction procedure ('DIVERSE', 'Rarefaction'), where the expected number of species (or in our case taxonomic richness) is calculated for a given number of individuals sampled (sample size). Taxonomic richness was estimated repeatedly for increasing sample sizes in each site, at increments of 5 individuals, until the total number of invertebrates collected within a given site was reached. If an asymptote in expected taxonomic richness is not reached for a given site, it suggests that actual taxonomic richness is higher than that estimated from the sampling effort.

Two-factor Analysis of Variance (ANOVA) was used to examine differences in abundance of earthworms, potworms, springtails, and Acari (mites) among sites and between sampling dates (seasons). Linear regression was used to test for correlations between abundance of these taxonomic groups and plant cover, filter media organic matter, and root biomass.

The contribution of invertebrate communities to nine important rain garden functions (plant growth, water infiltration, plant pathogen removal, denitrification, nutrient uptake in plants, nutrient storage in soil, heavy metal uptake by plants, coarse organic matter shredding, and decomposition) was estimated as follows. Briefly, the average abundance (from this study) of each taxonomic group capable of performing a function (Supplementary material, Table S1) was multiplied by a biomass correction factor based on body size. These values were summed and then multiplied by the number of contributing taxa present in our samples, positively weighting richness. The resultant scores were binned logarithmically so that function scores spanning multiple orders of magnitude could be compared (see Supplementary material, Appendix S1 for calculations).

A Monte Carlo framework (Mehring et al., 2015) was used to further evaluate a subset of the above-noted invertebrate functions in rain gardens concerning nitrogen. Specifically, we quantify (a) the percentage of total soil N in the tissues of earthworms, potworms, and springtails, (b) the amount of soil media ingested by dominant soil invertebrates (earthworms and potworms) in a single year, and (c) areal rates ($m^{-2}\,h^{-1}$) of N_2O and N_2 emission from earthworms in each rain garden (see Supplementary material, Appendix S2 for calculations). This approach required use of 1) our measurements of taxon-specific abundance and biomass from