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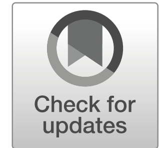

Evaluating the effects of canine urine on urban soil microbial communities

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Abstract

Due to extensive areas of impermeable surfaces, combined sewer overflow (CSO) is currently a major problem in urban areas across the United States. In CSO systems, sewage can travel through underground pipes to be decontaminated in treatment facilities, or it can combine with stormwater after a precipitation event and discharge into local waterways. Many cities are implementing green infrastructure installations, which use vegetation and bioactive soil microbial communities to enhance soil water-holding capacity, thereby minimizing CSO events. Understanding the factors that structure soil microbial communities in green infrastructure will facilitate more effective management of these engineered ecosystems; however, few studies to date have evaluated ecological patterns and processes of microbes in the urban environment. Nitrogen loading is known to be a major factor structuring fungi and bacteria in non-urban soils, and since cities also contain large populations of canines, N-rich urine deposition is a potential factor that could be important for structuring soil microbes in ground-level green infrastructure installations. Our study investigated the effects of canine urine on the urban soil microbial communities in a greenhouse experiment by treating *Liriope muscari,* a common plant found in New York City green infrastructure, with different concentrations of canine urine for 4 weeks in an experimental setting. We found that urine application significantly decreased total soil microbial biomass and microbial richness, and increased water runoff volume. These findings indicate that canine urine may have negative consequences for soil water-holding capacity and nutrient cycling in urban green infrastructure installations by directly decreasing the abundance and richness of soil microbial communities.

Keywords Green infrastructure · Canine urine · Nitrogen cycling · Soil microbial communities · Microbial diversity · Stormwater management

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Introduction

More than half of the world's population lives in urban environments and numbers are only expected to grow (McDonald et al. 2008; Wu 2014; Bocquier 2014). Increased urbanization over the past century has caused a dynamic shift from natural vegetated surfaces to increased levels of impermeable surfaces, such as concrete and asphalt, also known as grey infrastructure. Grey infrastructure impedes the natural percolation of water through soil, which is challenging for urban stormwater management, especially in cities with combined sewer overflow (CSO) systems. Following a precipitation event, water that fails to be absorbed by the soil is combined with raw sewage and overflow portions are released into nearby waterways, such as the Hudson River in New York City (Gómez-Baggethun and Barton 2013). This phenomenon contributes to water pollution, flooding, and eutrophication in

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aquatic ecosystems along streams and watersheds (Carter and Jackson 2007; Gómez-Baggethun and Barton 2013). Higher runoff volumes due to excess stormwater also induce strain on the underground pipes and these systems require continual maintenance and upgrade. A cheaper and more sustainable alternative to expanding the sewer system is to implement green infrastructure (GI), which are vegetated spaces in the urban environment such as tree pits, parks, and bioswales. These green spaces are spatially efficient solutions for minimizing CSO events as they transform impervious surfaces to permeable, vegetated areas (Villarreal and Bengtsson 2005), which reduce stormwater volume, delay peak discharge of water runoff into nearby watersheds and wastewater treatment plants, filter pollutants collected by stormwater runoff, and recharge groundwater (National Research Council 2008; Oberndorfer et al. 2007).

While the benefits of GI installations on stormwater management are widely recognized, these vegetated habitats also provide co-benefits for maintaining the diversity of urban biota, which contribute to the ecosystem services these engineered ecosystems are valued for. The majority of ecological studies of GI biodiversity focus on plants; however, soil microbial communities are also vital for the functioning of GI installations, as they facilitate the formation of soil aggregates that increase water-holding capacity, drive nutrient cycling to maintain plant growth and nutrition, and catabolize organic contaminants that may otherwise runoff in stormwater (Bellows 2001). Although the abiotic and biotic factors known to structure the diversity and function of microbial communities have been studied in non-urban environments, little research has been conducted on the urban soil environment, despite the potential to manage these belowground communities to optimize GI function (Xu et al. 2014; Ramirez et al. 2014; Gill et al. 2017).

Nitrogen loading is often elevated in urban ecosystems, which may negatively impact soil microbial composition and function, thereby reducing the functionality of GI communities (Treseder 2008; Kirchmann et al. 2013). In addition to atmospheric sources of nitrogen from fossil fuel combustion, one frequently overlooked input of nitrogen in urban soil is through the deposition of canine urine. Canines coexist with humans in high numbers in urban environments and frequently urinate on ground-level GI installations. As of 2012, New York City alone reported 600,000 documented domestic dogs and likely thousands of additional undocumented dogs, which translates to a significant quantity of daily nitrogen loading on public ground-level GI installations such as tree pits and parks (StatsBee 2012). While nitrogen is a key nutrient in microbial and plant growth and the richness of nutrients in urine makes it a good fertilizer at low doses (Pradhan et al. 2008), enhanced concentrations of nitrogenous compounds may result in adverse effects on soil systems including disturbances in soil osmotic regulation, acidification, and nutrient imbalance (Rooney et al. 2006). In addition, bactericidal effects are thought to be associated with the formation of nitrous and nitric acid at high nitrogen concentrations (Mancinelli and McKay 1983). Excessive nitrogen addition has been shown to inhibit microbial growth and abundance via leaching, denitrification or volatilization—loss of nitrogen as free ammonia (Clough et al. 1996; Broadbent 1965).

The effects of N-loading from domesticated animal urine has been found to negatively affect soil ecosystems in several studies of pasture animals. For example, in one study evaluating the effects of bovine urine, microbial biomass was significantly reduced in pasture soils following urine addition and nitrates were leached within the second week of urine treatment with no signs of soil recovery (Petersen et al. 2004). In a similar experiment, bovine urine treatment initially led to a peak in microbial activity, suggesting a nitrogen fertilization effect, but drastically fell a week later (Orwin et al. 2010). In another study on the effects of sheep urine addition, researchers found higher levels of soil surface pH, osmotic stress, and altered structures of soil bacterial communities (Singh et al. 2009; Shand et al. 2002). Changes in soil pH can alter the solubility of minerals and nutrients, which in turn affects the availability of nutrients taken up by plants, and is one of the most important factors known to structure soil bacterial communities (Lauber et al. 2009). Osmotic stress induced by urine addition can further change microbial communities by changing composition and decreasing microbial biomass by disrupting energy and nutrient flow (Singh et al. 2009). Although these various studies have shown mostly detrimental effects of pasture-raised animal urine on soil ecosystems, there is a lack of research on the effects of canine urine, specifically in urban environments.

For the current study, we evaluated the effects of canine urine on soil microbial composition, soil pH, soil microbial biomass and soil water-holding capacity using a concentration gradient of urine applied to potted plant species commonly found in NYC urban GI. We hypothesized that canine urine would be a source of nitrogen fertilizer upon initial application, but that the microbial diversity, microbial biomass, and water-holding capacity of the soil would decrease at higher urine concentrations. Findings from this study will improve future GI management in public spaces and provide a better understanding on urban nutrient cycling processes.

Materials and methods

Dog urine

We reached out to multiple dog adoption shelters and pet stores within the five boroughs of NYC and New Jersey to ask for permission to collect dog urine at their facilities; however, only one shelter consented. Despite visiting the shelter

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twice a week for couple months, less than 40 mL of urine was collected due to difficulty in predicting when the dogs will urinate and the dogs' refusal to continue urinating when a collecting bowl was brought near them as they were about to urinate. As an alternative, artificially manufacturing dog urine was also considered, but not enough dog urine was collected to assess its composition. We, therefore, used coyote urine purchased from AuSable Brand (Oscoda, MI) because it was commercially available, and coyotes are in the family Canidae and closely related to domestic dogs.

The amount of urine applied to each soil sample was determined by the average amount a dog urinates during a single event, which was estimated based on average dog body weight and average daily urine volume according to previously published studies (Kendall et al. 1982; Michel and King 1997). NYC's top five most popular dog breeds of 2015 were French Bulldog, Bulldog, Labrador Retriever, Golden Retriever and Yorkshire Terrier, and they continue to be popular in other major cities in the United States (Feeney et al. 2015; Cacich 2018). The five breeds' average body weight range from 2.5 kg to 30 kg (PetPrescription 2005). On average, dogs urinate 10 to 40 mL of urine per kg of body weight in a day at least three to five times each day (Osborne 2003; Cesar's Way 2015). Assuming that each dog urinates 25 mL per kg of body weight per day, the median value, and urinates five times a day, one event of urination of a 19 kg dog produces approximately 95 mL of urine. For this study, the total treatment solution volume was reduced to 75 mL to account for some of the missed urine that end up on the sidewalk or the GI's fence instead of on the soil when dogs urinate near GI installations.

Sample treatment and soil processing

To evaluate the effects of canine urine addition on soil microbial composition and soil holding capacity, we added a urine concentration gradient to potted *Liriope muscari* (Decne) L.H.Bailey (Asparagaceae) plants, an herbaceous plant commonly planted in GI installations across New York City. A total of 125 samples of *Liriope muscari* (*L.muscari*) were obtained from Classy Groundcovers (Blairsville, GA), and were grown in individual plastic pots (4 in. in diameter and 3.75 in. deep) containing water trays with urban soil obtained from New York Recycling LLC. This soil source is the same as that used to fill many GI installations around the city (NYC Parks Department, personal comm.). The composition of the soil was 72% sand, 18% silt and 10% clay. Plants were grown in an incubator room at ambient temperature (15–25 °C) using fluorescent grow lights with light intensity 726.56 ~ 871.88 $\mu E\ m^{-2}\ s^{-1}$ that were installed at the top of each shelf of the incubator unit. On the first week of treatment, all samples were given 75 mL of water every day. To compare how different concentrations of urine affected soil microbial communities and to find the threshold of change, concentrated coyote urine was serially diluted with water and applied to the *L. muscari* samples using a unispense machine every day for the remaining 3 weeks in a urine concentration gradient of 0%, 25%, 50%, 75% and 100%. Each solution treatment totaled to 75 mL per day.

Weekly destructive sampling was conducted to analyze five samples from each urine concentration group, totaling 25 plants per week. Soil samples from the potted plants were collected in sterile Whirl-Pak bags. Each sample was passed through a 2 mm sieve, that had been sterilized with ethanol and 15 min of UV radiation, to homogenize the soil. The total volume of water runoff produced from each sample was measured in a graduated cylinder and used as a proxy for the water-holding capacity of soil (Arshad et al. 1996).

Soil pH was measured on air-dried soils with a glass electrode in 1:2 water at the conclusion of the experiment.

Illumina sequencing

For fungal and bacterial analyses, DNA was extracted from 0. 25 g subsamples using PowerSoil® DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. The first internal transcribed spacer region (ITS-1) in fungi was amplified with primer pairs ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS-2 (GCTGCGTTCTTCATCGATGC) (McGuire et al. 2013). The V4 hypervariable region of the 16S bacterial rRNA gene was amplified with primer pairs: 515-F (AATGATAC GGCGACCACCGAGATCTACAC) and 806-R (CAAGCAGAAGACGGCATACGAGAT) (Caporaso et al. 2011). Both the forward and reverse primers were modified to include the appropriate Illumina adapters and sample-specific index sequences on the reverse primer. All DNA samples were amplified in duplicate PCR reactions containing 10.5 μL water, 12.5 μL GoTaq Master Mix, 0.5 μL each of the forward and reverse primers, and 1 μL genomic DNA. PCR reactions were completed at 94 °C for 3 min, 60 °C for 1 min and 72 °C for 2 min for 35 cycles. Products of the PCR reactions were pooled and observed on an agarose gel and quantified using the PicoGreen dsDNA assay. Pooled amplicon libraries were sequenced on a single Illumina Miseq lane by the New York Medical Center Genomics Core Laboratory (Valhalla, NY).

Reads from the sequencing were de-multiplexed, filtered to remove low quality reads with a maximum e-value of 1, and processed using QIIME to obtain sequences with an average of no more than one base error per sequence (Caporaso et al. 2010). Sequences were clustered at 97% similarity to identify operational taxonomic units (OTUs), using the UPARSE pipeline (Edgar 2013) and mapped on representative phylotype sequences to generate an OTU table. OTUs were assigned taxonomic names using the Ribosomal Database Project database. OTU tables, taxonomic assignments and sample data

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were imported into R using the phyloseq package (McMurdie and Holmes 2013). Sequence abundances in each OTU table were rarefied to 2350 sequence reads for 16S and 1540 reads for ITS prior to further analyses.

Phospholipid fatty acid analysis

Fungal and bacterial biomass was measured using phospholipid fatty acid analysis (Frostegård and Bååth 1996). Soil samples were freeze-dried in a lyophilizer (Freezone 2.5 Liter Freeze Dry System, Labconco, Kansas City, MO) for 48 h and phospholipids were extracted from 2 g of soil subsamples using a 1:2:0.8 mixture of chloroform, methanol and citrate buffer. Lipids were separated into neutral, glycolipid, and phospholipid fractions using a 3 mL silica solid phase extraction column and adding 2 × 2.5 mL chloroform. The phospholipid fractions were collected by adding 2 × 2.5 mL of methanol into the silica columns and drying the extract under N_2 gas at 40 °C. The collected phospholipid fractions were transesterified into fatty acid methyl esters (FAME) using 1 mL of 0.2 M KOH and CH_3OH solution per sample since every microbe has its own FAME fingerprint (Hill et al. 2000). FAME in the samples were identified by comparing the retention times of a bacterial acid methyl ester standard using the gas chromatography system (Agilent 6980 N, Agilent Technologies, Santa Clara, CA). To identify retention times of peaks, internal standards 13:0 and 19:0 were used. Peaks for 28 different microbial biomarkers (Table 1) were selected in nmol values and later converted to units of microbial biomass (nmol/g of dry soil). Total PLFA, corresponding to total microbial biomass, was calculated by summing the values from the 28 identified biomarkers. Bacterial to fungal biomass ratios were calculated by dividing nmol/g of dry soil values for the total value of selected bacterial biomarkers (i15:0, a15:0, 15:0, 10Me16:0, i17:0, a17:0, 17:0, 16:1ω7t, 18:1ω7, cy19:0) by the fungal biomarker (18:2ω6,9).

Table 1 28 Fatty acid methyl esters (FAME) fingerprints used to determine total microbial biomass and bacterial to fungal biomass ratio

FAME	Name
2-OH 10:0	2-hydroxydecanoate
2-OH 12:00	dodecanoic acid, 2-hydroxy
3-OH 12:00	dodecanoic acid, 3-hydroxy
i14:0	iso-tetradecanoic acid
14:0	tetradecanoic or myristic acid
i15:0	iso-pentadecanoate
a15:0	anteiso-pentadecanoate
15:0	pentadecanoate
2-OH 14:0	tetradecanoic acid, 2-hydroxy
3-OH 14:0	tetradecanoic acid, 3-hydroxy
16:1ω7c	10-cis hexadecenoic acid, 10Z-hexadecenoic acid, 16:1(n-10), 16:1delta10
16:1ω7t	9-trans-hexadecenoic acid, palmitelaidic acid, E-9-hexadecenoic acid, trans-palmitoleic acid
i16:0	iso-hexadecanoate
16:1ω9	cis-9-Hexadecenoate, palmitoleic acid
16:0	Me. Hexadecanoate, palmitate, Hexadecenoic acid
10Me16:0	Hexadecanoic acid,10-methyl; 10Me16:0 fatty acid; 10-Methyl-hexadecansaeure; 10-methyl-hexadec
i17:0	15 methyl hexadecanoate
a17:0	anteiso-heptadecanoic acid
17:0	heptadecanoic acid or margaric acid
2-OH 16:0	hexadecanoic acid, 2-hydroxy
18:2ω6,9	Linoleic acid, cis,cis-9,12-octadecadienoic acid, 9z,12z octadecenoic acid, 18:2(n-6), c18:2n6c
18:1ω9c	cis-9 octacdecanoate, Oleic Acid Methyl Ester
18:1ω9t	trans-9 octadecanoate, Elaidic Acid Methyl Ester
18:1ω7	Octadecenoic Acid, Vaccenic Acid (cis & trans combined?)
18:0	octadecanoate, stearic acid, methyl stearate
cy19:0	cis-10 nonadecanoic acid
19:0	nonadecanoic acid
20:0	eicosanoate or eicosanoic acid

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Statistical analyses

Data were analyzed using R (v. 2.7). Bray-Curtis measure of similarity was used to calculate pairwise distances in abundances among the sample groups due to time and concentration. The relative abundances of OTUs were square root transformed prior to analysis. We used non-metric multi-dimensional scaling (NMDS) plots to visualize clustering patterns in the overall microbial community and the statistical significance of clustering patterns were determined using analysis of similarities (ANOSIM). Additional NMDS plots were separately configured for bacterial and fungal communities to further visualize differences in microbial communities across urine treatments. Soil microbial richness, evenness and diversity were analyzed using one-way analysis of variance (ANOVA) in R. Changes in the water-holding capacity of the system was analyzed by measuring the mean volume of water runoff and normalizing the average volume of water runoff of the control. Plots for water runoff and community relative abundance were also generated in R.

Results

DNA sequencing revealed that bacterial community composition was significantly impacted by both urine concentrations and the duration of urine application, whereas fungal composition only differentially responded to the latter. Shannon diversity across samples with canine urine treatment was negatively correlated with time in bacterial communities, decreasing more than one third by week two (Fig. 1c), but the Shannon diversity remained relatively unchanged in fungal communities across treatments (Fig. 1b and d). Additionally, shifts in bacterial community composition due to duration of urine application were apparent with the gradient of increasing urine concentrations. Relative ratios of Firmicutes to Proteobacteria in the bacterial community significantly increased with increasing urine concentrations and time. Between the first 2 weeks of urine treatment, the percentage of Firmicutes changed less than 1% for the control samples, increased about 56.67% for the 50% urine concentration samples, and increased over 90% for the 100% urine concentration samples (Fig. 1a).

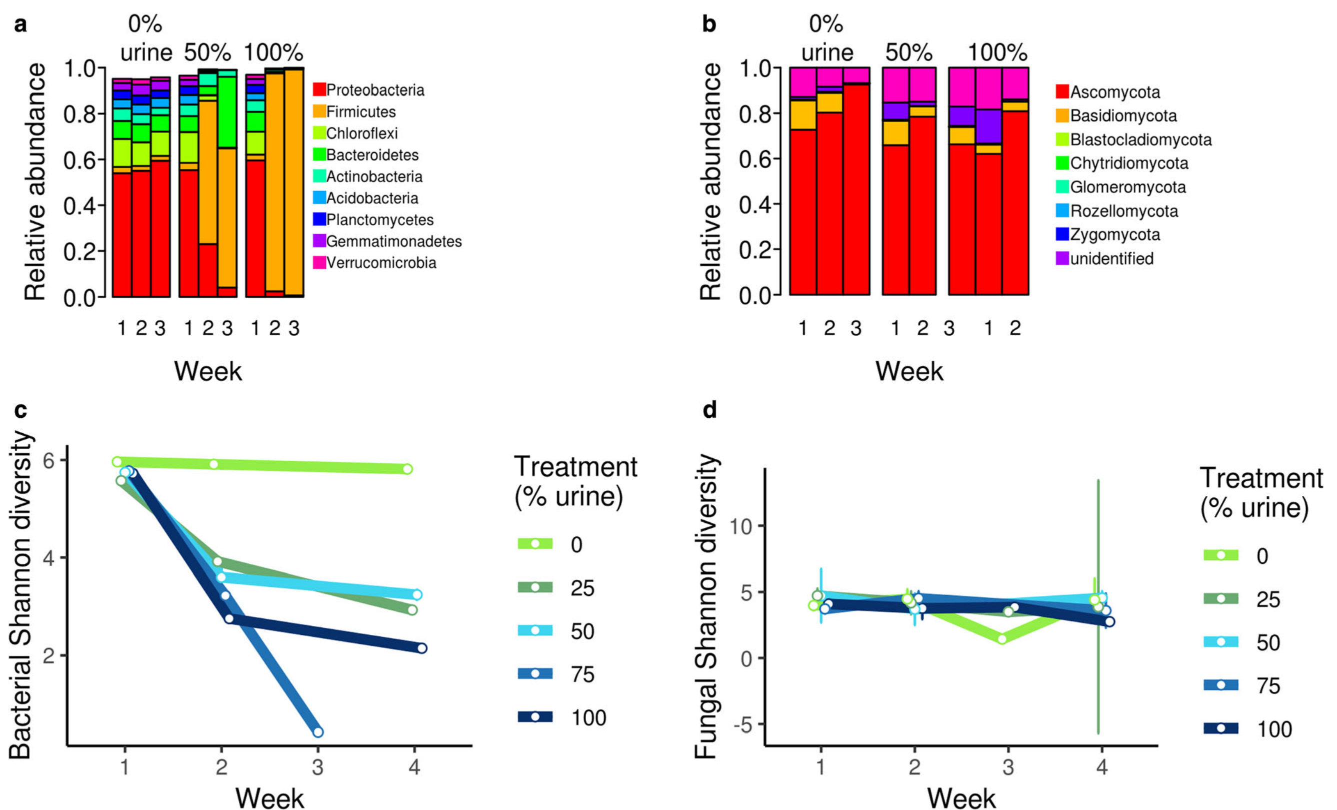

Fig. 1 Comparisons of microbial diversity affected by urine concentration and urine application duration: **a** bacterial phyla, **b** fungal phyla, **c** bacterial Shannon diversity, and **d** fungal Shannon diversity. Microbial diversity was negatively impacted by addition of urine treatment. Bacterial communities were significantly impacted by both urine concentrations and duration of urine application, whereas fungal communities only differentially responded to duration

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Phospholipid fatty acid analysis indicated that there was a general decrease in total microbial biomass and bacterial biomass throughout the experiment. For both the total microbial biomass and bacterial biomass, there was an initial peak for the 50% urine concentration group at week two but the peak dropped more than five-fold by week three (Fig. 2a). Furthermore, the bacterial to fungal biomass ratio in all treatment groups initially decreased eight-fold in week two, but a general increase in the ratio was observed by the end of the experiment (Fig. 2b).

The volume of water runoff significantly increased over time for the experimental groups treated with canine urine (Fig. 3b; $F_{2,122} = 3.537$, $P = 0.032$), but not for the control plants. The decrease in mean water volume in the 25% urine concentration sample group can be due to outliers in data due to small sample size, or it may suggest that the microbes were able to use nitrogenous inputs as a fertilizing source that becomes detrimental to the system after prolonged application. Relative to the control group, samples treated with urine generally exhibited greater runoff volume (Fig. 3a).

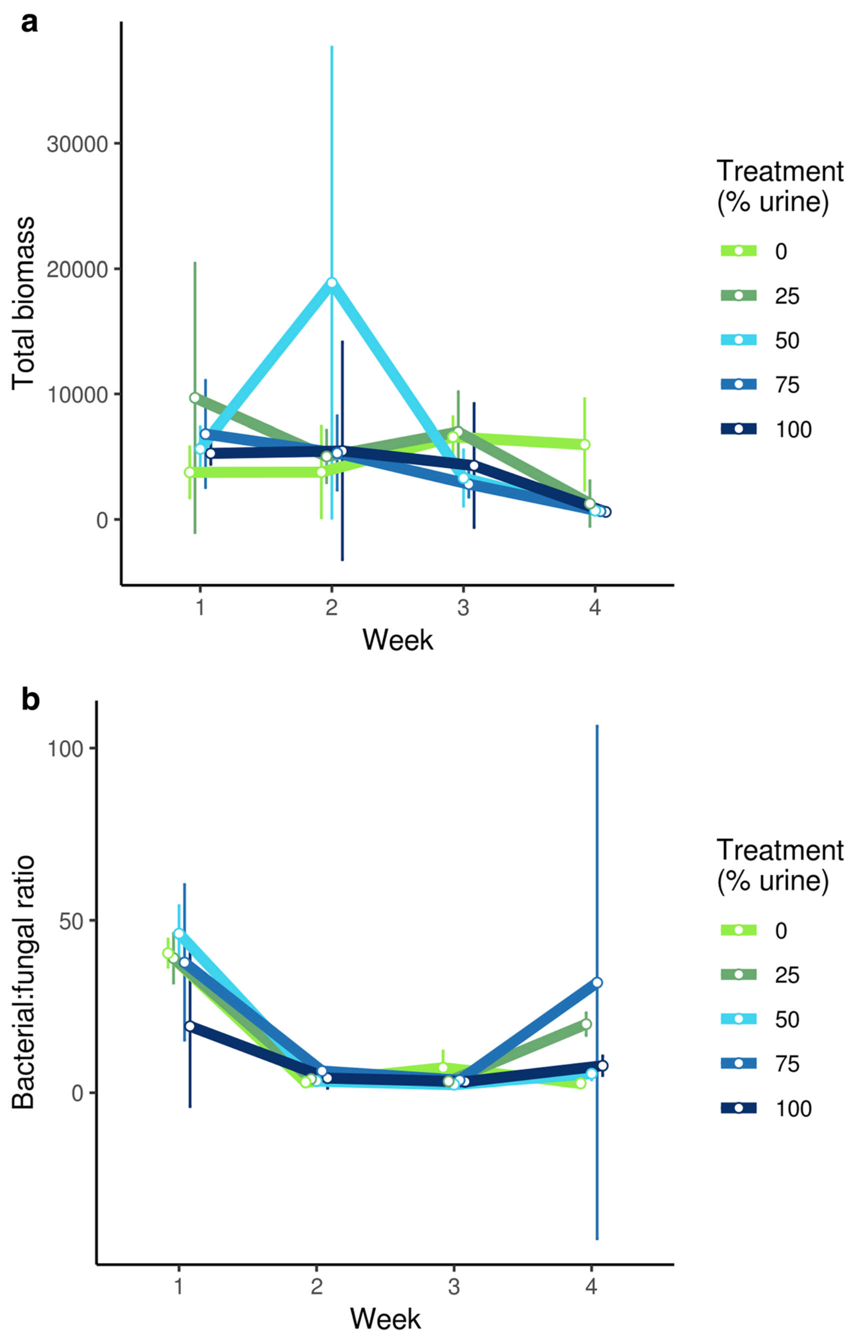

Fig. 2 Changes in total soil microbial biomass (**a**) and bacterial biomass to fungal biomass ratio (**b**) by treatment. Microbial biomass decreased throughout the experiment and showed no signs of recovery. Bacterial: fungal biomass ratio decreased eight-fold in week two, but a general increase in the ratio was observed by the end of the experiment

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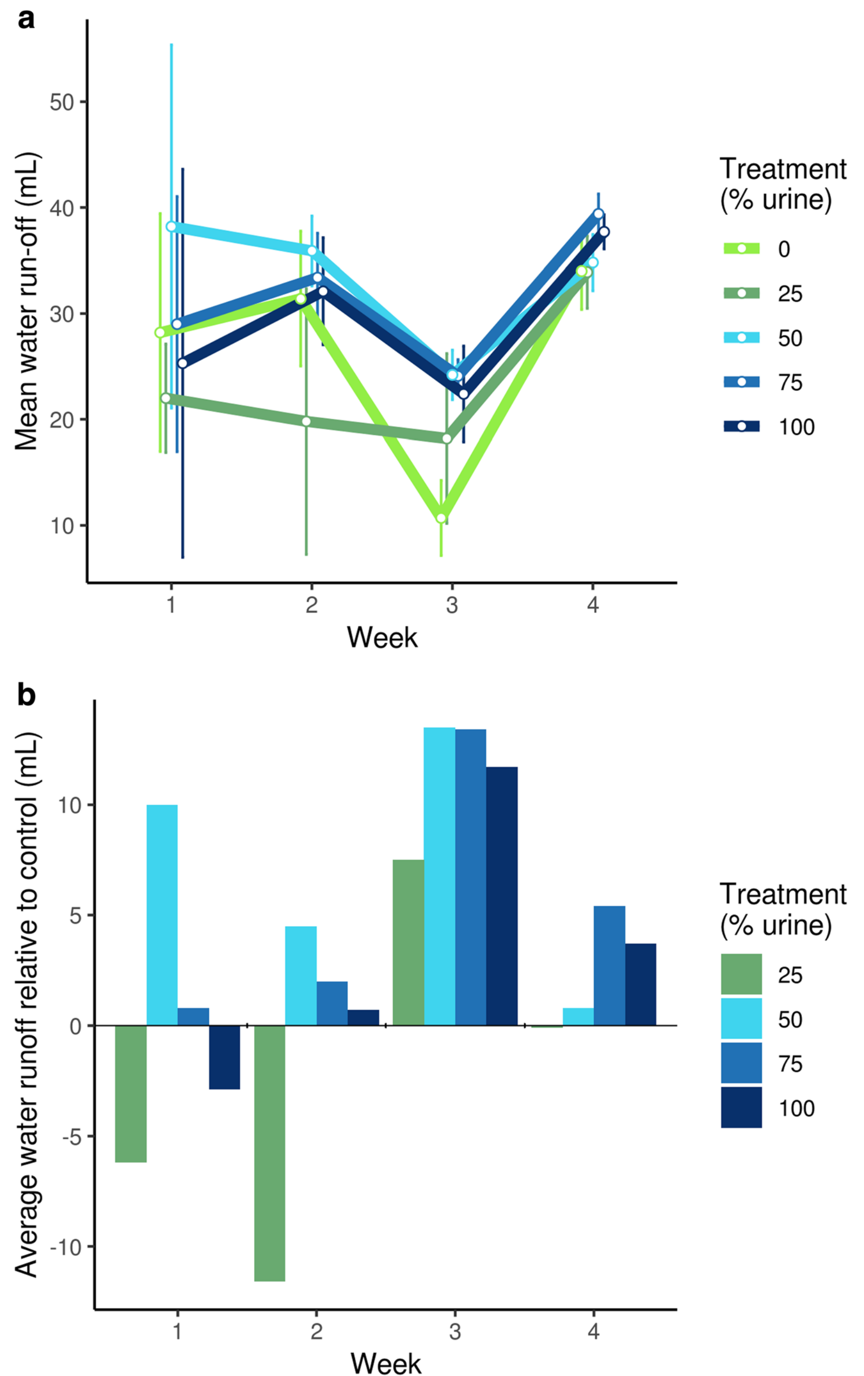

Fig. 3 Changes in average water runoff volume (**a**) and average water runoff volume relative to the control (**b**) by treatment. Stormwater runoff volume increased over time for the experimental groups treated with urine

There was a significant decrease in soil pH across the experimental groups treated with canine urine relative to the control group, and pH decreased with increasing concentration of urine application (Fig. 4). Bacterial metrics of Shannon diversity and phylogenetic diversity were positively correlated with increases in soil pH (Fig. 5a and c). Shannon diversity and fungal richness were also positively correlated with increases in soil pH, although the relationship was weaker for fungi than for bacteria (Fig. 5b and d).

Discussion

Canine urine constitutes a significant source of nitrogenous input to urban soil environments, and our data suggest that even small quantities of urine can negatively impact soil microbial communities over short periods of time. Previous studies have shown that the accumulation of ammonia and ammonium in soil from excess nitrogenous inputs can be detrimental to both microbial communities and plants by increasing the ionic strength of the surrounding soil and osmolarity, thereby

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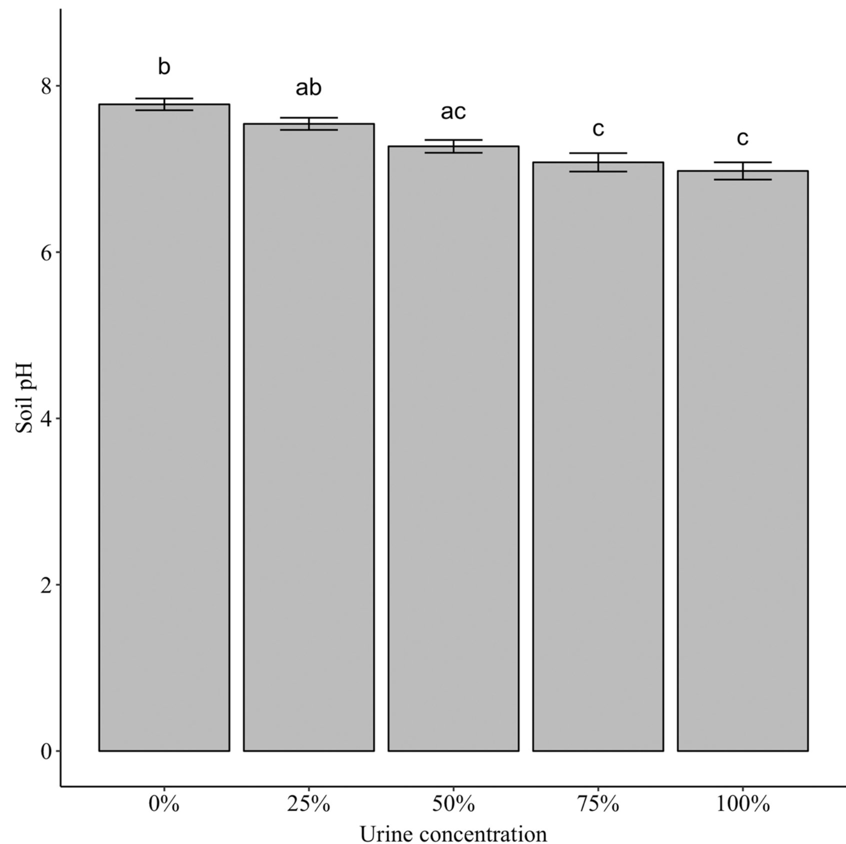

Fig. 4 Changes in soil pH, as measured at the end of the experiment, across all urine treatments

pulling water away from the organisms and dehydrating them (Müller et al. 2006; Orwin et al. 2010; Singh et al. 2009; Williams et al. 1999). Similarly, we found that microbial changes in response to urine were significantly related to urine-induced changes in soil pH.

Results of our study showed negative correlations between microbial diversity and canine urine concentration, and between microbial diversity and treatment duration, suggesting that canine urine inputs can have rapid, negative consequences for soil microbial communities. As expected, microbial diversity of both fungal and bacterial communities in the control samples remained relatively unchanged, indicating that microbial responses at the end of the experiment were not due to growth chamber conditions. Microbial diversity decreased in all samples treated with urine, even at the lowest urine concentration, highlighting how even small inputs of urine can have significant effects on soil microbial composition. Based on an investigation of the impact of nitrogen fertilizer on grassland soil microbial community diversity (He et al. 2013), the decrease in bacterial diversity in our study may also be attributed to the visually noticeable reduction in root biomass of the *Liriope* plants in response to urine deposition. Using Shannon and Simpson indices, He et al. (2013) reported a reduction in both microbial functional diversity and belowground plant biomass in soils that were treated with high nitrogen fertilizers over a 3 month period. Plant roots exude various chemicals into soil that act as signals to initiate symbiosis between plants and microbes in the rhizosphere, promoting soil microbial activity and diversity (Bardi and Vivanco 2009; Zhong et al. 2010). Although the present study did not measure the changes in belowground plant biomass, the root volume decreased conspicuously after urine application for all treatment groups. Besides releasing chemicals that facilitate plant-microbe symbiosis, large root biomass also benefits vegetation by minimizing dehydration as large root volumes buffer the exposure of roots to ammonia (Williams et al. 1999). Progressive scorching in our *L. muscari* samples suggests that the plants failed to experience such buffer effect, resulting in inefficient nutrient recycling by soil microbial communities and further immobilization of nitrogen (Williams et al. 2000).

In addition to changes in microbial diversity, composition also shifted, and the dominant bacterial phyla changed from Proteobacteria to Firmicutes with increasing urine concentration over the duration of the experiment, which may be explained by functional differences in survival abilities unique to each phylum. While the majority of Firmicutes can form endospores that allow them to survive in unfavorable environmental conditions, Proteobacteria lack this ability and thus die in extreme environments (Murray and Baron 2003). Furthermore, a relative decrease in the diversity of the Proteobacteria phyla can potentially reduce important functional microbes, such as nitrifying bacteria. Nitrifying bacteria convert soil ammonia and ammonium to nitrite or nitrate, and because ammonia is corrosive, its accumulation in soil renders

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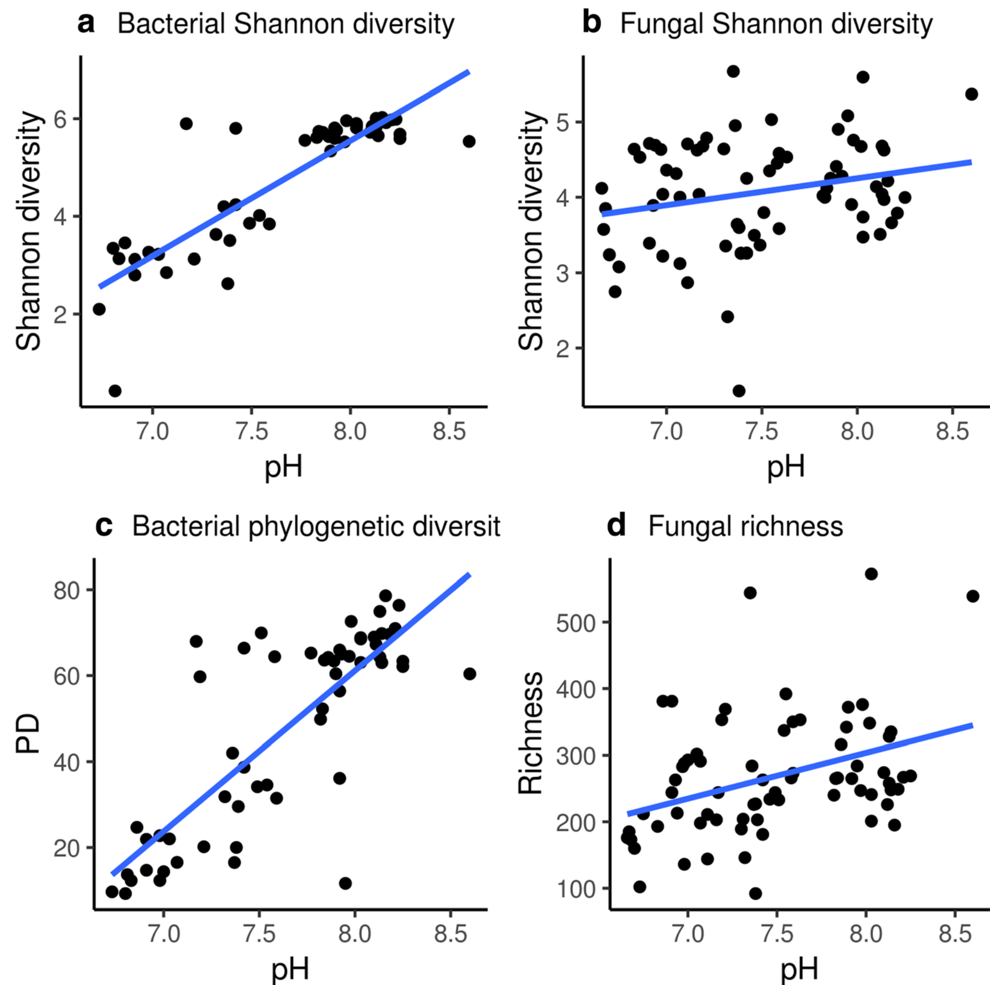

Fig. 5 Relationships between final soil pH values and bacterial Shannon diversity indices (**a**), fungal Shannon diversity indices (**b**), bacterial phylogenetic diversity (**c**), and fungal richness (**d**). Since the hypervariable ITS region was amplified for fungi, phylogenetic diversity estimates could not be calculated, so OTU richness is presented

it highly toxic to both plants and microbial organisms (Murray and Baron 2003; Hovanec and DeLong 1996). In fact, excessive nitrogenous compounds such as nitrates and ammonium have been found to decrease soil pH (Michael 2018), which is what we observed in the current study. Soil pH is known to be one of the most significant factors shaping microbial communities globally (Fierer et al. 2009; Lauber et al. 2009), so it is likely one of the dominant factors that caused shifts in bacterial communities in this study. Fungi also respond to pH at local and regional scales, although the strength of the relationship is weaker than for bacteria (Rousk et al. 2010), which is also what we observed in this study. In an urban area with large populations of canines, the accelerated rate of soil acidification due to an exogenous input of nitrogen may lead to the removal of important microbial groups that function to maintain healthy soil environments.

In contrast to the noticeable shift in bacterial community composition, the fungal community was largely dominated by the Ascomycota phylum throughout the experiment, suggesting that this fungal phylum was not as negatively affected by urine application as others. The relative stability of fungal groups across decreasing levels of soil pH show that they may be better at adapting to changes in acidity than bacterial groups are, and have wider growth tolerances to pH changes, as has been observed in other systems (Rousk et al. 2010).

Our results indicated an overall decreasing trend in microbial biomass throughout the study in experimental groups treated with canine urine, demonstrating that urine application not only changes the composition of soil microbes, but also decreases their overall abundance. An initial peak in total microbial biomass and bacterial biomass at week 2 was observed only in the 50% concentration group, although with high variability across samples. This result may have been due to the outliers from small sample size or increased microbial growth and activity for some communities due to an initial fertilization effect, as has been observed in previous studies using bovine and sheep urine (Orwin et al. 2010; Williams et al. 1999). Another potential explanation could be that the relatively more acidic environment produced by urine application favored fungal growth. Bacterial groups were likely disadvantaged by lower soil pH, thereby decreasing the competition with fungal groups (Rousk et al. 2009). However, we speculate that the increasing bacterial to fungal ratios found towards the end of the experiment indicated that fungi may have been

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damaged by excess nitrogen after prolonged exposure. Although fungi are generally better at scavenging and recycling nitrogen than bacteria, they also display greater sensitivity to high nitrogen deposition (Treseder 2008).

Throughout the experiment, we observed a significant increase in the volume of water runoff; if this same pattern occurs in the urban environment following canine input, GI effectiveness at capturing stormwater may be reduced with frequent dog visitations (Bellows 2001). The mechanism for this phenomenon was not uncovered but could be related to the decrease in soil microbial biomass, as this metric is often related to soil water holding capacity (Bellows 2001; Murphy et al. 2011; Rawis et al. 2003). It is also possible that key microbial taxa were eliminated, which contribute to vital microbial functions and processes such as nutrient cycling as well as filtrating and storing water (Chaer et al. 2009). Instability in this soil system due to the reduction in functional diversity from urine treatment reiterates the need to reduce additional nitrogenous input in urban soils. If our findings are broadly applicable to in situ conditions, reductions in nitrogen usage may decrease the volume of stormwater runoff channeled to sewer systems in cities during precipitation events and consequently minimize CSO events since nitrogen leaching from fertilization applications was found to be greater in flooded soils (Mandic et al. 2013).

In a densely populated city like NYC, taking into account illegal dog breeding and the myriad of unregistered dogs—only 20% of the estimated 530,000 dog population were licensed in 2006 (Wilkinson et al. 2006)—there is likely an even more significant amount of daily nitrogen loading than official documentation may suggest. While local ordinances (such as section 1310 of the New York State Public Health Code) are implemented to maintain dog control with respect to the proper removal of dog feces by the person possessing custody of the dog, little to no enforcement exists to regulate dogs from urinating in public (New York Department of Sanitation 2009). Furthermore, current GI installation designs often lack structures that physically protect the installations from exposure to dog visitation. Ideally, an irrigation system or capture and filtration system should be implemented in urban environments where dogs frequently visit to redistribute small amounts of excess nitrogen to soils as a nutrient-fertilizing source through dilution. Our rough calculations based on previously published quantities of N in canine urine as a function of dog size (Kendall et al. 1982; Michel and King 1997) revealed that an average dog weighing 25 pounds can contribute up to 17.9 kg N ha − 1 y − 1, which is nearly double the amount from atmospheric nitrogen (Harada et al. 2018). This suggests a tremendous external source of nitrogen input from canines alone.

Our study provides insight into the detrimental effects of excessive nitrogenous input via urine deposition on urban soils, and to better understand microbial feedbacks on plant community dynamics, future studies should evaluate the effects of varying concentrations of urine deposition on different combinations of soil and plant species in situ. Previous studies have found that the use of nitrogen fertilizers resulted in a depletion of soil carbon, which is an important nutrient for fungal growth and activities (Allison et al. 2010). Thus, additional research should be performed on the differential rates of nitrogen and carbon cycling to study the role of urban microbes in regulating the biogeochemical cycle of nutrients vital for plant growth and survival. Since increased levels of soil nitrogen greatly affected the ability of microbial communities to support the lifespan of plants, we highly suggest further implementation of engineered green infrastructure installations to support robust soil microbial communities, that account for N inputs from dog visitation.

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