The influence of vegetation type on runoff nutrient concentrations from an extensive green roof system

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ABSTRACT

Green roofs, also known as vegetated roofs, have been shown to provide a wide range of environmental benefits in urban areas. However, runoff from green roofs may contain soil nutrients like nitrogen and phosphorus, as well as dissolved carbon, which are contaminants if present in excess in water. These contaminants degrade water quality, and as a result can lead to nutrient enrichment problems downstream. This study compares concentrations of total nitrogen, nitrate, and dissolved carbon in the runoff from a modular, extensive green roof system among different vegetation types. Additionally, a supplementary analysis of the growing media was assessed for nitrate, phosphorus and potassium levels. Given that plant species differ in their nutrient requirements, identifying species with high nutrient demands could help reduce runoff nutrient concentrations. Analysis of runoff was done both prior to, and following the addition of a controlled-release fertilizer. Results from runoff nutrient analysis show that, for both total nitrogen and nitrate, vegetation type had a strong effect. Treatments with overall higher amounts of living biomass, such as species mixtures and monocultures of *Sedum acre,* resulted in better runoff quality (i.e. lower nitrogen concentrations) than those with little to no biomass. A regression between biomass and runoff nutrient concentrations suggests that nutrient uptake is affected in part by biomass production in green roof systems. Selection of higher biomass species or species mixtures can help improve the environmental performance of green roofs.

April 24, 2019

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1. INTRODUCTION

1.1 Green roof structure, classification, and usage

A green roof, or vegetated roof, refers to an area of a roof that is either partially or completely covered by vegetation. This vegetation is usually supported by a number of engineered layers to promote plant growth in an area which natural plant cover is not usually found. These layers typically consist of (from top to bottom), vegetation, substrate (growing medium), a filter/drainage layer, root barrier, and some type of waterproofing membrane (Vijayaraghavan & Joshi, 2015) (Figure 1).

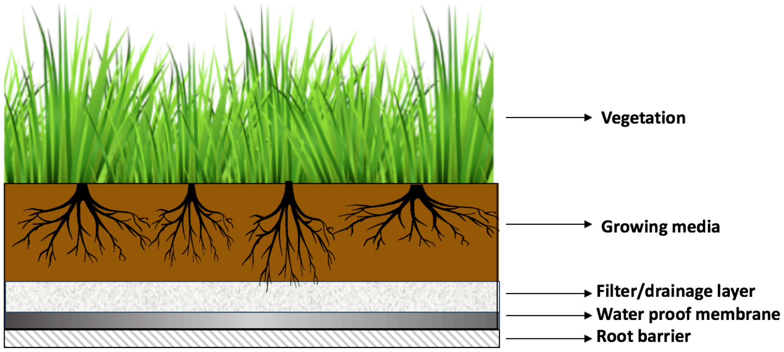

Figure 1. Schematic representation of multi-layered green roof cross section.

The morphology of a green roof typically categorizes it in one of two structural categories: an intensive or extensive green roof. Intensive roofs are comprised of deeper substrate depths, usually measuring 30 cm or more (Berndtsson *et al*. 2008). This depth allows for the growth of larger vegetation such as trees or shrubs. Intensive roofs are also referred to as “roof top gardens” as they function primarily in creating amenity space, and consist of vegetation that is generally more visually appealing (Berndtsson *et* *al.* 2008).

Extensive green roofs are comprised of shallower substrate depths, usually measuring no more than 10-15cm (Berndtsson *et al*. 2008; Whittinghill *et al*. 2014). This shallower depth limits vegetation types to smaller growing plants such forbs, grasses and succulents, in particular *Sedum* spp. (Berndtsson *et al*. 2008; Whittinghill *et al.* 2014; Withers *et al.* 2014). Extensive green roofs, although not as aesthetically pleasing as intensive green roofs, are typically constructed for their production of both environmental, and associated economic benefits (Berndtsson *et al.* 2005; Rowe, 2011). Such benefits include increased habitat for animals living in urban ecosystems (Berndtsson *et al.* 2005; Buttschardt, 2001), energy conservation related to the regulation of building temperature (Berndtsson *et al.* 2008, Buffam *et al.* 2016), mitigation of air and noise pollution (Berndtsson *et al.* 2008; Whittinghill *et al.* 2014) and reduction of urban heat island effects (Berndtsson *et al.* 2005; Berndtsson *et al*. 2008). The appearance of green roofs is not a recent event, and dates back long before modern urbanization and industrialization occurred (Berndtsson *et al*. 2005; Monterusso *et al.* 2005). However, as these processes continued to expand and evolve to what they are today, the appearance of green roofs has begun to grow with them.

The addition of greenery to an otherwise industrialized area is what gives green roofs their aesthetic, and even therapeutic appearance (Berndtsson *et al.* 2005). In addition to their aesthetic value, there are a variety of reasons for the application of green roofs in urban environments, and their functional importance should not be overlooked. The installation of green roofs in urban areas function in mitigating the effects of pollution that result from heavy industrialization (Rowe, 2011). Moreover, one of the most notable environmental benefits provided by green roofs is their storm water retention capability (Berndtsson *et al.* 2005).

Unlike non-vegetated roofs, green roofs allow for retention of storm water within their substrate. This degree of retention varies depending on a number of factors including the depth of substrate used, substrate moisture content prior to rain, slope of the roof, rate of evapotranspiration, local climatic conditions, and vegetation morphology (Berndtsson, 2010; Rowe, 2011). While many studies have shown reduction of runoff through use of a vegetated roof (Berndtsson *et al*. 2010, Johannessen *et al.* 2017, VanWoert *et al.* 2005.), their results are not directly comparable to one another as the amount of water retained is highly variable. This variability of water retention depends on several factors such as the experimental methods used to measure water retention, the amount of precipitation applied, the type of vegetated roof, and duration of the experiment (Berndtsson, 2010). Climatic seasonal variation can also influence the water retention capacity of green roof systems. For example, a study by Johannessen *et al.* (2017), looked at the water retention performance of green roof systems in Northern Europe along a climatic gradient. Their findings showed that water retention was around 25mm in cold and wet locations, but increased to 40-50mm in warmer and dryer locations (Johannessen *et al.* 2017).

In general, however, green roofs can retain anywhere from 50-100% of storm water from a single precipitation event (Rowe, 2011). Storm water capture can be beneficial for cities in low-lying areas which may suffer from frequent flooding, as well as the prevention of metal contaminants from non-vegetated roofs collected by runoff entering water drainage systems (Clark *et al.* 2008).

1.2 Green roof runoff and water quality

Despite the increased number of studies looking at storm water retention and reduction of runoff, few have closely looked at the composition of green roof runoff (VanWoert *et al*. 2005; Berndtsson, 2010). There is currently much still unknown regarding runoff composition, and in particular, nutrient concentrations within the runoff. The type of growing medium used on a green roof varies based a number of ecological and structural factors that carry certain limitations. For example, the loading-bearing capacity of a roof, as well as the type of construction materials used may limit the amount and type of substrate applied. From an ecological standpoint, certain vegetation types have different requirements for the type of growing medium used based on both physiological and morphological characteristics. It is likely that the growing medium used will contain a mixture of minerals derived from materials such as shale, sand, or clay, as well as nutrients such as phosphorus, nitrogen, and potassium to promote optimal plant growth (Peter *et al.* 2010). Compost is a common component within the growing media of green roofs due to its nutrient content that helps establish and support plant growth. Regardless of fertilizer added to the substrate, nutrients found within the compost can leach out, leading to runoff with relatively high nutrient concentrations compared with runoff from conventional roofs. This can lead to eutrophication (i.e. nutrient enrichment) problems downstream, making enrichment from nutrient runoff a potential ecosystem disservice of green roofs. As a result, green roofs are beginning to be studied as a potential source of water pollution due to degradation of water quality from their runoff (Berndtsson *et al*. 2005; Buffman *et al.* 2016).

1.2.1 Soil leaching and fertilizer application

Similar to ground-based plants, plants on a green roof require nutrients for optimal growth. The need for nutrients is perhaps even greater on a green roof, as the vegetation is growing in more extreme conditions. For example, overcoming more environmental stressors (depending on the height of the roof) such as increased UV exposure, higher wind speeds, and more extreme temperatures fluctuations (Lükenga & Wessels, 2001). It is therefore no surprise that newly constructed green roofs are often heavily fertilized, especially during the first year to promote rapid plant growth, establish plant communities, and ultimately add aesthetic value. However, this heavy application of fertilizer can lead to a large input of nutrients into the substrate, such as phosphorus and nitrogen. Despite the plants' needs for these nutrients, over-fertilization can leave excess nutrients stored in the substrate (Ju *et al.* 2004).

Nitrogen (N) is a crucial element required for healthy plant growth. It has important biochemical and physiological roles that are involved in the metabolic processes of plants. Such processes include the production of proteins and chlorophyll - a major component of the photosynthetic pathway: the process through which plants convert sunlight into a usable (chemical) energy source (Leghari *et al.* 2016). Despite its abundance in the atmosphere, plants cannot use the inert, atmospheric form of nitrogen (N_2) in their metabolic processes (Leghari *et al.* 2016). Instead, ions such as nitrate (NO_3^-) and ammonium (NH_4^+) are forms of nitrogen which are easily taken up by plants, and are therefore used in most fertilizers to yield a high growth rate (Heim & Lundholm, 2014). While NH_4^+ is more easily adsorbed by soil particles, NO_3^- is left mobile and not bound by other compounds or abiotic factors (Bin-LeLin *et al.* 2001; Ju *et al.* 2004). If nitrate accumulates to excess in the substrate, it may be leached out via water drainage, thereby entering runoff from the roof (Bin-LeLin *et al.* 2001; 17). Other ions, such as phosphates, have a more complex chemical relationship with soil, and are typically more readily adsorbed (Zak & Gelbrecht, 2007). Adsorption of phosphorus ions does not allow them to pass as freely through soil particles with the passage of water as do nitrates. Phosphorus compounds can, however, still be leached and enter the runoff (Schmieder *et al*. 2018). Although beneficial to plants in the soil, nutrients like nitrate and phosphate are considered hydrological contaminants, which intensifies if accumulated in watersheds.

1.2.2 Eutrophication

Eutrophication is the excessive enrichment of water from nutritive substances, and is a natural, ecological process that occurs in bodies of (usually fresh) water (Schindler, 2006; Vijayvergia, 2008). This process often results from plant-derived nutrients (such as phosphorus and nitrogen) entering bodies of water via runoff, and in turn poses a number of environmental threats (Chislock *et al.* 2013). Unfortunately, eutrophication is often a result of anthropogenic activities that accelerate this process, leading to excess algal and plant growth that reduce oxygen levels in the water, and ultimately disrupt aquatic ecosystems (Chislock *et al*. 2013). As both terrestrial and aquatic agricultural practices expand due to an increasing demand from the growing global human population, the need for high yield of crop production pushes for excessive fertilizer application (Withers *et al.* 2014). This makes eutrophication a growing environmental concern. Agricultural practices, however, may not be the only substantial contributor to more frequent incidents of eutrophication. As green roofs are appearing more frequently, there is an increased opportunity for leaching of nutrients from the substrate into the runoff, and subsequently entering water ways. The current lack of research surrounding nutrient concentrations in green roof runoff makes it difficult to predict if vegetated roofs are contributors to degraded water quality in both urban and aquatic ecosystems. While it is not known if they represent a significant contribution to increased incidents of eutrophication, it is pertinent to know what environmental effects could arise based on the concentration of nutrients within green roof runoff.

1.3 Study organisms

Vegetation types used on a green roof are variable, and also limited by factors such as local climatic conditions, green roof structure, and the resources (plant species) available in that region. Presently, there is research looking at which plant species, or mixture of species, function better on a green roof in producing the desired environmental benefits (Heim & Lundholm, 2014; Lundholm, 2015). There is, however, limited research comparing runoff composition between vegetation types. This study uses a variety of common ruderal species (representing different vegetation types) found in Nova scotia. The vegetation types used in this study are often found on lawns around the province, particularly in Halifax, where this study was conducted. The species used are currently colonizing the library green roof on Saint Mary's University campus, and have been placed in a modular green roof system that has been established for five years. As a result, these species have shown to be suited to green roof conditions.

1.4 Research objectives

This study aims to develop a better understanding of the runoff nutrient content produced from vegetated roofs. The main objective of this study is to fill the research gap regarding nutrient concentrations in the runoff of a variety of vegetation types when placed in an extensive green roof system. Previous work using a similar green roof system showed nutrient concentrations, especially nitrate, were quite low in the growing medium after four years (Lundholm, 2015). As a result, a fertilizer-addition experiment was conducted in order to assess the plant's response to the addition of nutrients in the growing medium. As nutrient concentrations of the growing media should be higher after fertilizer application, it was hypothesized that the fertilized treatments of this experiment will show better differentiation in runoff nutrient concentrations (i.e. nitrogen and dissolved carbon) than the unfertilized treatments among different vegetation types.

2. METHODS

2.1. Experimental design and Set up

This study was conducted on the library green roof of Saint Mary's University in Halifax, Nova Scotia (44°39'N, 63°35'W). The extensive, modular green roof system that was used was initially set up in June 2014. It consisted of 360 x 360 x 120 mm deep trays with a lattice of holes at the base to allow free drainage (Anderson Die-Deep Propagation Flat; Stuewe and Sons, Tangent, OR, USA). In this study, one tray constitutes one module.

Each module contained three constructed green roof layers, including a composite water-retention mat fitted at the base (Huesker, Charlotte, NC, USA), followed by an

Enkamat (a plastic webbing over a root barrier (Colbond, Enka, NC, USA)). The top layer (substrate) contained 60 mm of green roof growing media (Sopraflor X; Soprema, Drummondville, QC, Canada) (Lundholm et al. 2010)). Sopraflor X is a mixture of expanded shale, blond peat, perlite, sand, and vegetable compost that has a total porosity of 50-60% and a bulk density of 1200-1300 kg/m^3.

Modules were arranged in a block design consisting of six blocks that were two modules wide and 10-12 modules long (Figure 2). Each module was placed in contact with as many other modules as possible to reduce edge effects, but still allowed space for walk-ways (Lundholm *et al*. 2010). A total of 130 modules were used, consisting of thirteen experimental treatments, each with ten replicates. The treatments were set up as follows: eight monocultures (each a different species); one *Sedum spp.* monoculture (planted 2017); two mixtures (all species excluding *Sedum,* and one excluding clover); one spontaneous vegetation mixture (composition uncontrolled; growing medium only, with no subsequent weeding); and one control treatment (bare substrate; growing medium only, with all plants removed periodically (weeding: see below)). Nine individual plants of roughly the same size were transplanted from the library green roof into a module, evenly spaced apart in a square grid. Mixture treatments contained one individual from each species with no particular arrangement. Only eight species were used in the mixture treatments, therefore a duplicate of one of the species was rotated through the replicate modules as the ninth individual. Treatments were randomly arranged throughout the blocks, with similar replicate numbers from each treatment grouped together. Treatments were controlled by weeding new growth of unplanted species biweekly and prior to sampling. Aside from natural rainfall events, no supplemental irrigation was used during the sampling period (May-August 2018).

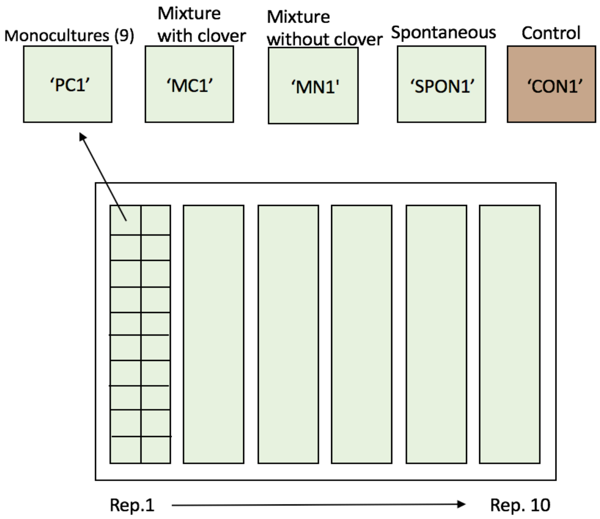

Figure 2. Schematic representation of the block design set-up of the green roof system. Six narrow blocks evenly spaced apart, containing 20-22 modules in each block, each containing 1-2 replicates from each treatment. The five boxes above represent the treatments used in the experiment.

Figure 2.1. One of the blocks used in the experiment.

2.1.1 Plant Species

The vegetation in this experiment consisted of a variety of common lawn weed species (mostly non-native) that represent four different life-form groups. The four groups include grasses: *Poa compressa* L. *(Pc);* forbs: *Cerastium fontanum (Cf), Ranunculus repens* L. *(Rr), Taraxacum officinale (To), Trifolium repens* L. *(Tr), Veronica serpyllifolia* L. *(Vs), Hieracium flagellare (Hf);* tall forbs: *Plantago major* L. *(Pm);* and succulents: *Sedum acre L. (Sa).* These species were growing naturally (not planted) on the library building green roof, and are commonly found on lawns around Halifax. Consequently, they have been found to colonize similar green roof systems throughout the city, and have therefore been shown to be suited to green roof environments.

2.2 Canopy density

A non-destructive index of the above-ground living biomass was obtained for every module by measuring canopy density. Canopy density index was estimated through use of a three-dimensional pin frame (Domenico Ranalli, Regina, SK, Canada) using the point interception method (Jonasson, 1988). The frame measured 30cm x 36 cm x 36 cm, and contained 16 rods evenly spaced apart, each 6mm in diameter (Figure 3). For the purpose of this study, canopy density is considered as the number of contacts between a pin frame rod and live plant part per m^{-3} (Lundholm, 2015). To obtain an index value, the frame was placed on top of a module, and each time a piece of living biomass touched a rod it was given a value of one. The number of contacts for each module was totalled, giving a canopy density value for that module (Tran *et al.* 2018). If no biomass touched a rod, but individual plants were still present, the module was given a total value of one (Heim, 2013). Data were collected for all modules once a month for the duration of the experiment.

Figure 3. Pin frame used to collect canopy density index (Photo: ©Amy Heim, 2013).

2.3 Experimental Fertilizer Application

Since the initial application of growing medium in 2014 (all treatments except *Sedum acre*) and 2017 (*Sedum acre*), there has been no further application of growing media or fertilizers to any of the treatments. A study done by Lundholm (2015) showed that over a 4-year period, nutrients such as nitrate and phosphate in the substrate became reduced to very low levels. These nutrient concentrations are likely too low to distinguish variation among vegetation types, and may fall below detection thresholds of certain nutrient analyses. As this experiment focuses on analyzing differences in the nutrient concentrations as affected by different vegetation types, fertilizer was applied one week following the first round of sampling in mid-July. This was done to ensure an adequate amount of nutrients were available to detect vegetation differences in nutrient dynamics, in the analyses of the runoff and growing media. One ‘scoop’, equal to 30 mL, of fertilizer (Plant-Prod® Smartcote® ‘Perennial & Rose’ 12-12-12 with micronutrients controlled release fertilizer) was sprinkled evenly across each module in the top 1/4 inch of growing medium. Fertilizer was applied to 7 out of 10 replicates for each treatment, leaving three replicates unfertilized.

2.4. Runoff sampling

A pilot study was conducted on extra planted modules (not part of this experiment) prior to the first sampling event. The purpose was to determine the average time taken for runoff to concede, as well as determine the amount of water needed to obtain the minimum volume of runoff required for nutrient analysis. The results determined that the modules required a minimum of 2 L of water to produce 40 mL of runoff from each treatment for analysis. This pilot experiment followed the same sampling protocols used on the real study (outlined below). Runoff samples were collected on days with no precipitation to control the amount of water entering the modules.

Each module was placed on top of a plastic collection tray to allow water to drain from the base of the module and collect in the tray below (Figure 4). A watering can filled with 2L of hose water (tap water) was poured evenly over the surface of the growing media over a span of 30 seconds using a timer. This rate of precipitation equates to approx. 15 mm of rain in 30 seconds, or 30 mm of ‘rain’ per minute. The modules were then left for a period of 7 minutes to allow runoff to collect in the tray below. After this time, total runoff volume was measured using sterile glass beakers, and the value was recorded.

Immediately after, a sample of 40mL was collected from the runoff and placed in a labelled VWR® Polyethylene scintillation vial, with a sample of hose water taken as a control. Vials were sealed and stored at -20° centigrade until shipped offsite for analysis. This process was repeated for all 130 modules during each sampling event. Four separate sampling events took place over the duration of the experiment: two prior to the addition of fertilizer, June 21st and July 5th, and two following the addition of fertilizer, July 25th and August 21st. Runoff volume was recorded for all four events. Due to loss of physical data and time restraints for nutrient analysis, runoff samples for nutrient analysis were collected for only three events, one prior to fertilizer application, and two following fertilizer application.

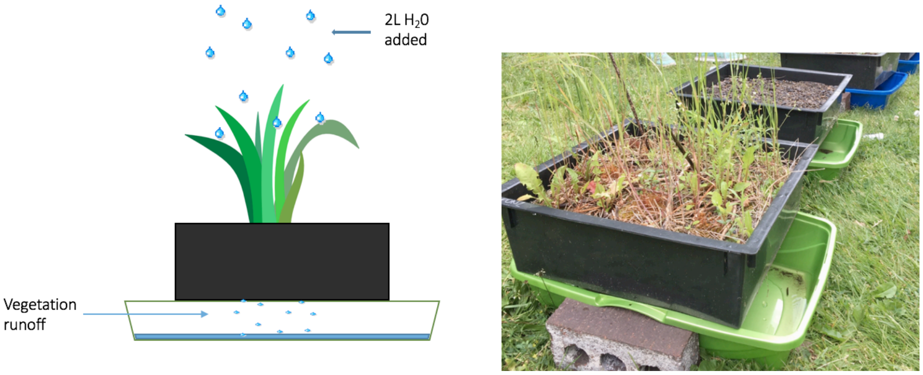

Figure 4. Schematic representation of the runoff sampling experimental design (left). Modules during the runoff sampling event (right).

2.4.1 Nutrient Analysis of Runoff

Nutrient analyses of the runoff were conducted on samples from every module for all treatments. Analyses included total organic carbon (DOC), total nitrogen (TN) and nitrate (NO_3). DOC and TN were measured using a Shimadzu TOC-VCPH analyzer, equipped with Shimadzu TNM-1 unit for the TN analysis. Nitrate was measured using a microplate adaptation (Ringuet et al. 2003) of the single-reagent vanadium chloride spectrophotometric method (Doane and Horwath, 2010). Analyses for each module were performed using the two vials of runoff; DOC and TN used one vial, and nitrate used the second vial. Analyses were carried out on unfiltered samples, which were preserved by freezing, then thawed immediately prior to analysis. For modules with added fertilizer, samples were diluted due to a matrix effect suppressing colour development for nitrate analysis. Samples were typically diluted 5:1 or 10:1 using pure milli-Q water. Samples from unfertilized modules were diluted only as necessary to obtain values appropriately within the standard range. All analyses were carried out by Nick Willse (Buffam Laboratory, Dept. of Biological Sciences, University of Cincinnati, 2018).

2.5 Nutrient Analysis of Growing Media

Six modules (three unfertilized, and three fertilized) from each treatment were used for nutrient analysis of the growing media one week following the end of the last sampling period (August 2018). Roughly 500 mL of growing media was removed from the sampled modules. Any macroscopic biomass (including roots), as well as fertilizer beads were removed by hand upon sampling. For the purpose of this study, samples were analyzed for nitrate (AOAC method 986.31, 15th Edition), as well as phosphate (P_2O_5) and potassium (K_2O) (Mehlich 3 extraction, run on Jarrell-Ash ICAP 9000) by the Nova Scotia Department of Agriculture Laboratory Services (Truro, Nova Scotia, Canada).

2.6 Statistical Analysis

All analyses were completed using the software program R and RStudio (1.1. 463) (R Core Team, 2018). All data sets were first tested to assess normal distribution using a Shapiro-Wilks normality test. If the data was not normally distributed, it was transformed using a logarithmic function to as close to normality as possible.

For all data sets, a mixed model ANOVA was used using treatment as the fixed effect and block as the 'random' effect. A mixed model was first run to determine if the block variable had any significant effects on the data. In most cases, the variance attributed to block was very low, with p values that were not significant ($p > 0.05$). To see if effects from a general 'random' variable had any influence on the data, a null 'random' factor was constructed, then compared against the first model that used block as a random effect. A two-way ANOVA of these two models showed the random effect in most cases to not be statistically insignificant ($p > 0.05$). In these cases, a simpler model (one-way ANOVA) was used. If the ANOVA produced a significant p value ($p < 0.05$), Tukey Pairwise Comparison tests were completed to assess which treatments differed significantly from one another.

In determining the relationship between canopy density and runoff nutrient concentrations, as well as total nutrient output, linear regressions were used. For some variables, data was transformed logarithmically to maximize the adjusted R-squared value. For most variables, line of best fit was constructed using an inverse equation ($y = 1/x$), otherwise a linear equation was used.

To compare both runoff nutrient concentrations, and number of pin hits over a period of time within a species, a repeated measures analysis was conducted, using ‘module’ as a repeated factor. This model takes into account any discrepancies within the data set attributable to any one specific module.

2.7 Precipitation rate calculations

The following equations were used to convert the amount of water added to each module into a precipitation rate:

[1] Convert surface area of module from m^2 to dm^2:

$$\text{module SA } (m^2) \times \frac{100\ (dm2)}{1\ (m2)}$$

[2] Determine amount (height) of precipitation in dm:

$$\frac{\text{volume of } H_2O\ (dm^3)}{\text{module SA } (dm^2)}$$

[3] Convert precipitation amount from dm to mm:

$$\text{precip. amt. } (dm) \times \frac{1000\ (mm)}{1\ (dm)}$$

3. RESULTS

3.1. Canopy Density

Application of a controlled release fertilizer had an effect on plant growth when measured over a period of time. Change in growth was detected statistically, but was also apparent visually (Figure 5). Results from a mixed model ANOVA showed that fertilized modules resulted in a significant increase in canopy density for monocultures of *P. compressa* (p = 0.0005274), *P. major* (p = 0.00125), and *S. acre* (p = 0.002644), as well as all three species mixtures ($p < 0.05$). (Table 1). Treatments that had a significant effect showed an increase in the number of pin hits (canopy density) as time went on. This result, however, is not the same for monocultures of *R. repens*, which showed a significant decrease in number of pin hits five weeks after fertilizer was applied (Table 1).

Table 1. Change in canopy density within a vegetation type over time. Repeated measures analysis using mixed model ANOVA. Canopy density measured as number of contacts between vegetation and pin. Values measured before fertilizer addition (Pre-fert) and after fertilizer addition (Post-Fert 1, Post-fert 2). Values represent least square means with standard error. Values with an asterisk* represent treatments with time having significant effect on vegetation growth (p <0.05). Values without shared letters are significantly different within a vegetation type only.

Vegetation Type	***Canopy density (pin hits/$0.18m^3$)***		
n =10	Pre-Fert	Post-Fert 1	Post-Fert 2
Control	**0**	**0**	**0**
C. fontanum	4 ± 1.5^{a}	4.29 ±1.27^{a}	2.29 ± 1.17^{a}
H. flagellare	11.6 ± 3.41^{a}	21.4 ± 2.49^{a}	14.4 ± 2.54^{a}
*P. compressa**	37.7 ± 7.64^{a}	41.7 ± 4.40^{a}	80.3 ± 7.91^{b}
*P. major**	4 ±1.18^{a}	9.29 ±1.73^{a}	22.29 ± 4.65^{b}
*R. repens**	16.6 ± 5.21^{ab}	25.7 ± 5.45^{a}	6.0 ± 1.23^{b}
*S. acre**	93 ± 19.24^{a}	120 ± 16.95^{a}	182 ± 9.46^{b}
T. officinale	14.9 ± 1.99^{a}	14.4 ± 1.99^{a}	23.1 ± 5.55^{a}
T. repens	13.57 ± 6.49^{a}	18.43 ± 8.13^{a}	8.57 ± 3.48^{a}
V. serpyllifolia	2 ± 0.82^{a}	1 ± 0.53^{a}	4.86 ± 3.03^{a}
*Mixture with clover (MC)**	49.6 ± 6.72^{a}	54.1 ± 3.65^{a}	79.1 ± 13.04^{b}
*Mixture no clover (MN)**	33.3 ± 1.99^{a}	49.7 ± 7.58^{ab}	59.3 ± 13.21^{b}
*Spontaneous (SPON)**	34.4 ± 3.52^{a}	58.6 ± 8.49^{ab}	65.0 ± 8.72^{b}

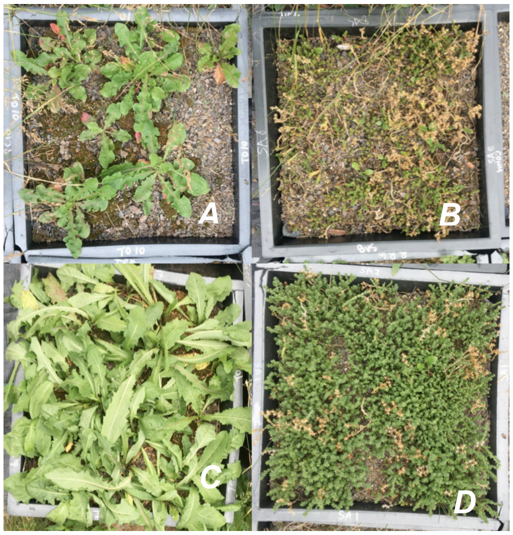

Figure 5. Modules with *T. officinale* (A) un-fertilized, (C) fertilized; *S. acre* (B) un-fertilized, (D) fertilized.

3.2. Runoff volume

A one-way ANOVA was used to estimate the effect of vegetation treatment on runoff volume. These results show that treatment had a significant effect on runoff volume prior to fertilizer addition (p = 0.0005063), and five weeks following fertilizer addition (p = 3.25e-05). Prior to adding fertilizer, the control treatment (i.e. no vegetation) had a significantly lower volume of runoff than other treatments, which all had higher runoff volumes, most of which did not differ significantly from one another (Figure 6A). Five weeks following the addition of fertilizer, it was found that monocultures of *S. acre* and *T. repens* produced significantly higher volumes of runoff than all other treatments (Figure 6B). Linear regressions were done to determine the relationship between canopy density (number of pin hits) and runoff volume. These results showed that the number of pin hits had no significant effect on runoff volume pre-fertilizer (p = 0.05215), two weeks post-fertilizer (p = 0.8219), or five weeks post-fertilizer (p =0.4532). Additionally, there was a very weak positive correlation between number of pin hits and runoff volume (R^2adj < 0.02).

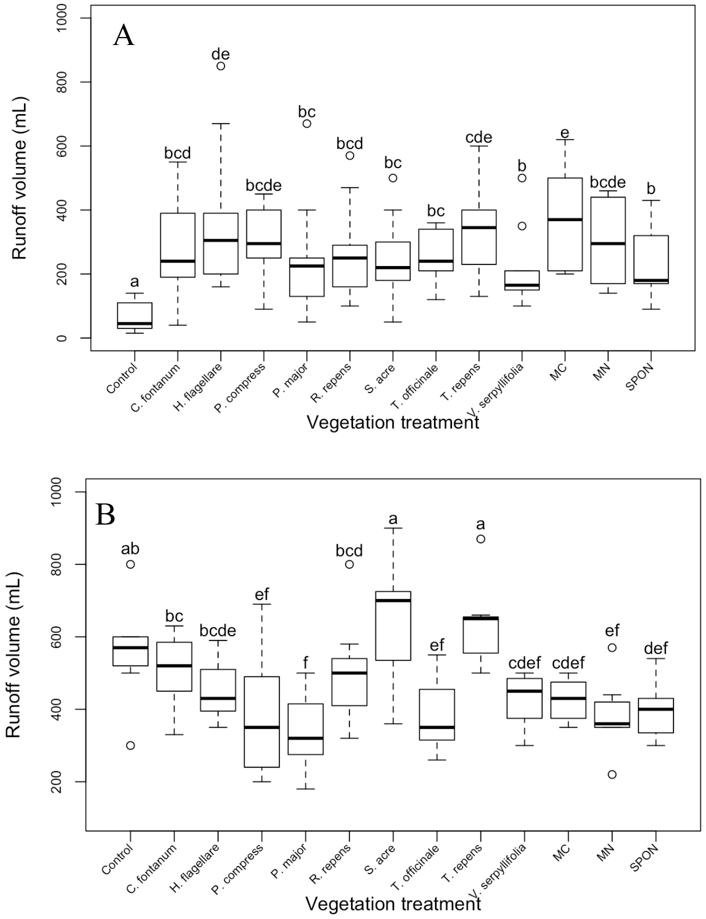

Figure 6. Box plots of the volume (mL) of green roof runoff (**A**) before fertilizer addition and (**B**) five weeks following fertilizer addition. Monoculture treatments indicated by Latin names, species mixtures indicated by identifier codes: mixture with clover (MC); mixture without clover (MN); spontaneous colonization (SPON). Boxes without shared letters are significantly different ($p < 0.05$).

3.2.1. Runoff nutrient concentrations

A Shapiro-Wilks test was used to assess if data were normally distributed. In most cases, data were transformed logarithmically so that they were distributed as close to normality as possible. A mixed model ANOVA was run using 'block' as random effect, and 'treatment' as a fixed effect. However, when compared against a 'null' factor, block effect was found to have low variance (0.014), indicating that the block likely does not have much of an effect on the data. Instead, a one-way ANOVA was used to estimate the effect of vegetation treatment on runoff nutrient concentrations. These results showed that vegetation treatment had a significant effect on runoff total nitrogen (TN) concentrations, as well as nitrate (NO_3) concentrations, both prior to and following fertilizer addition ($p < 0.05$). Prior to adding a controlled released fertilizer into the system, TN concentrations were low, ranging between 0.4 - 5.3 mg/L, with little variation among treatments (Figure 6). Following the fertilizer addition, levels of TN in the runoff increased, showing large variation among treatments. TN concentrations ranged from 3.3 - 107 mg/L, and 6.9 - 131 mg/L, sampled two weeks and five weeks post-fertilizer, respectively (Figure 7). Additionally, runoff concentrations of NO_3 showed similar patterns to TN. Pre-fertilized NO_3 concentrations ranged from 0.0 - 4.7 mg/L, with less variation among treatments (Figure 7A). Runoff concentrations of NO_3 also increased following fertilizer addition, and displayed larger variation among treatments. Nitrate concentrations ranged from 1.4 - 89 mg/L, and 4.4-123 mg/L, sampled two weeks and five weeks post-fertilizer, respectively (Figure 7B, C).

Overall, treatments such as species mixtures and S. *acre*, as well as a few other monocultures, had consistently low values of TN and NO_3 in the runoff. In contrast, monocultures of V. *serpyllifolia* and control treatments tended to have higher values.

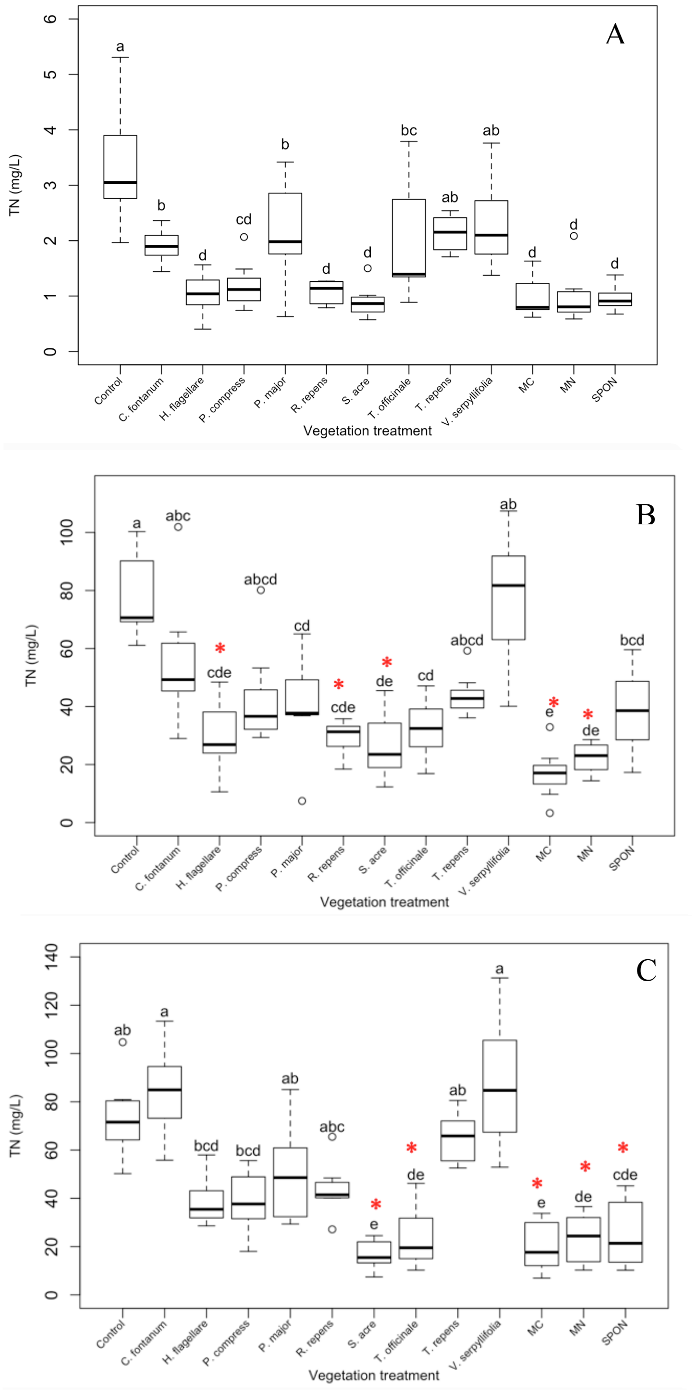

Figure 7. Box plots of runoff concentrations of total nitrogen (TN (mg/L)) sampled (A) prior to fertilizer addition ($F_{12,117} = 15.812$, $p = 2.2e\text{-}16$), (B) two weeks after fertilizer addition ($F_{12,78} = 9.8945$, $p = 2.024e\text{-}11$) and (C) five weeks after fertilizer addition ($F_{12,78} = 8.4339$, $p = 5.61e\text{-}10$). Monoculture treatments indicated by Latin names, species mixtures indicated by identifier codes: mixture with clover (MC); mixture without clover (MN); spontaneous colonization (SPON). Boxes with an asterisk* indicate the lowest total nitrogen concentrations. Boxes without shared letters are significantly different ($p < 0.05$).

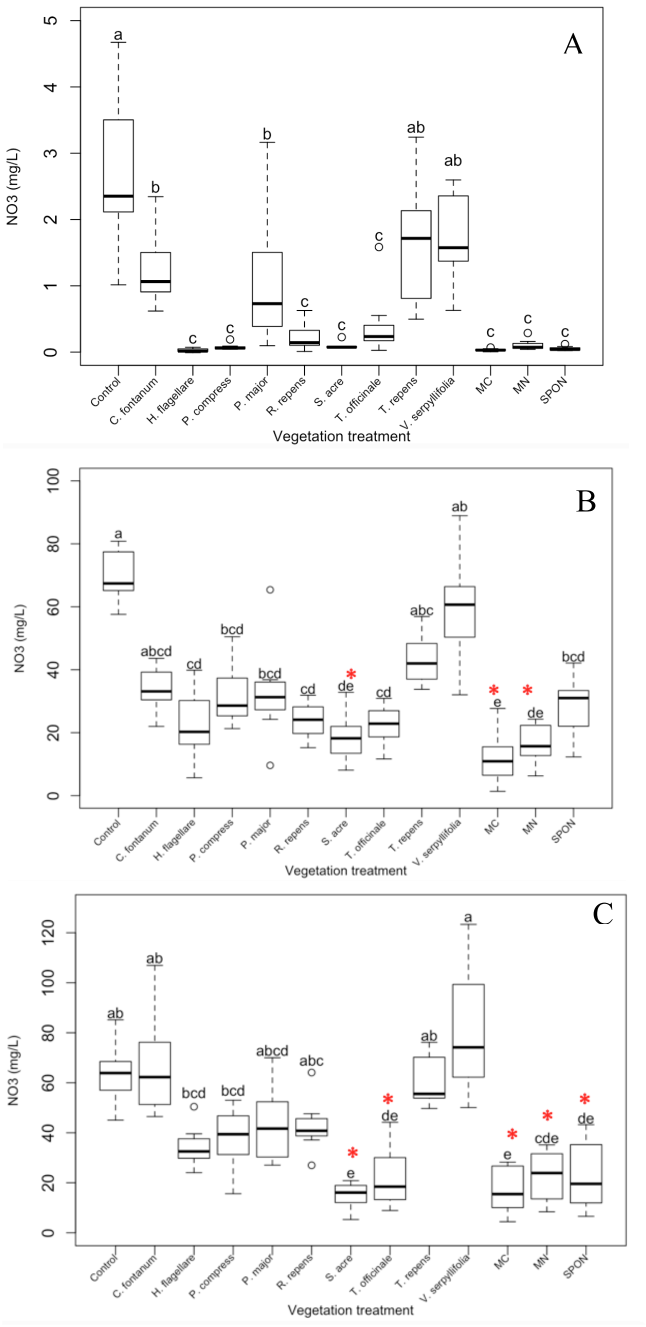

Figure 8. Box plots of runoff concentrations of nitrate (NO_3^- (mg/L)) sampled (A) before fertilizer addition ($F_{12,117} = 57.966$, $p < 2.2e\text{-}16$), (B) two weeks after fertilizer addition ($F_{12,78} = 31.839$, $p = <2.2e\text{-}16$) and (C) five weeks after fertilizer addition ($F_{12,\ 78} = 12.159$, $p = 1.881e\text{-}13$). Monoculture treatments indicated by Latin names, species mixtures indicated by identifier codes: mixture with clover (MC); mixture without clover (MN); and spontaneous colonization (SPON). Boxes with an asterisk* indicate the lowest total nitrogen concentrations. Boxes without shared letters are significantly different ($p < 0.05$)

Treatments that produced the lowest runoff nitrogen concentrations, overall, had more living biomass than treatments with higher runoff nitrogen concentrations. Such treatments included monocultures of *H. flagellare*, *P. compressa*, and *S. acre*, as well as all three species mixtures (MC, MN, SPON). Linear regressions were used to assess the relationships between biomass and runoff nitrogen concentrations. These results showed that biomass is in part attributable to low runoff nitrogen concentrations up to a certain point. Samples from before fertilizer addition, and two weeks post-fertilizer addition demonstrate a non-linear, inverse relationship between the number of pin hits and nitrogen concentrations (Fig. 9A, B). Samples from five weeks post-fertilizer demonstrate a negative linear relationship (Fig. 9C). For runoff total nitrogen (TN), all plots demonstrate an inverse relationship (Fig. 10).

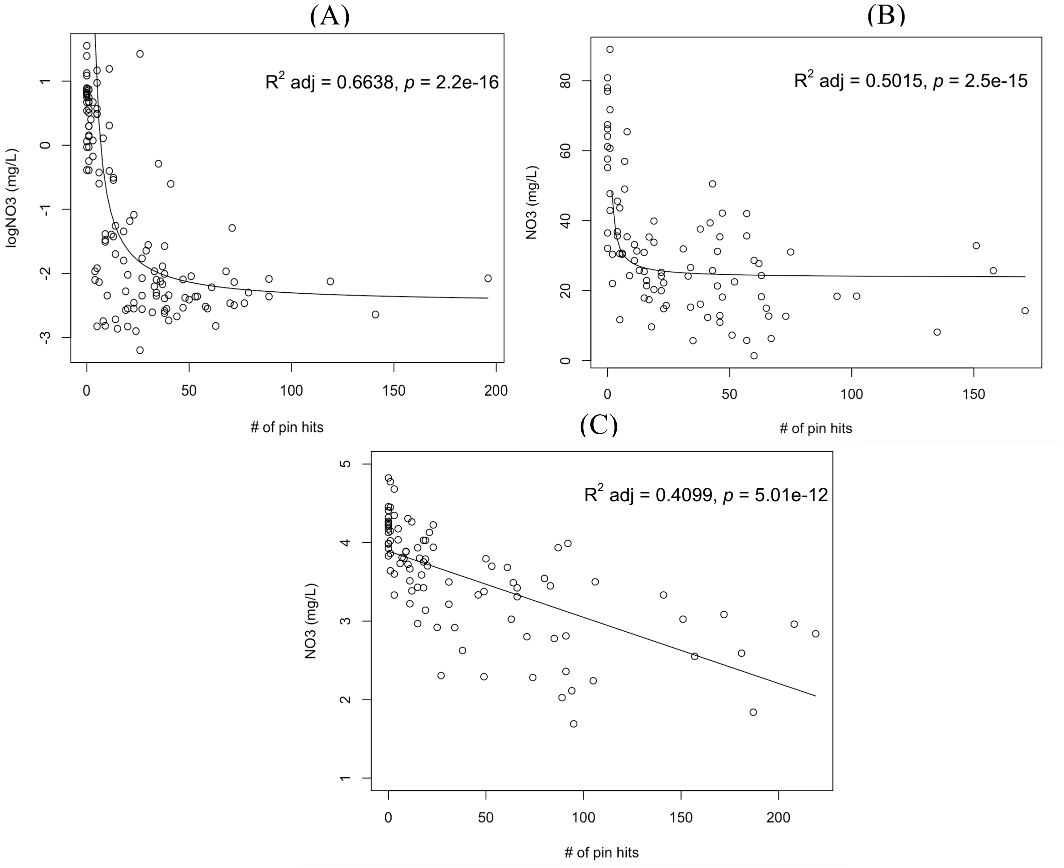

Figure 9. Linear regressions of runoff concentrations of NO_3^- (mg/L) compared to the number of pin hits for all treatments before fertilizer addition (n=130) and after fertilizer addition (n=91). **A**) Pre-fertilizer ($F_{1,128} = 255.7$, $p = 2.2e\text{-}16$). **B**) Two weeks post-fertilizer ($F_{1,89} = 91.54$, $p = 2.505e\text{-}15$). **C**) Five weeks post-fertilizer ($F_{1,89} = 63.52$, $p = 5.009e\text{-}12$). Lines of best fit are represented by inverse regressions (y=1/x) (A, B) or linear regressions (C), of transformed variables to maximize the R^2 –adjusted value. Pre-fertilizer plot shows transformed data (A); post-fertilized plots show raw data (B, C).

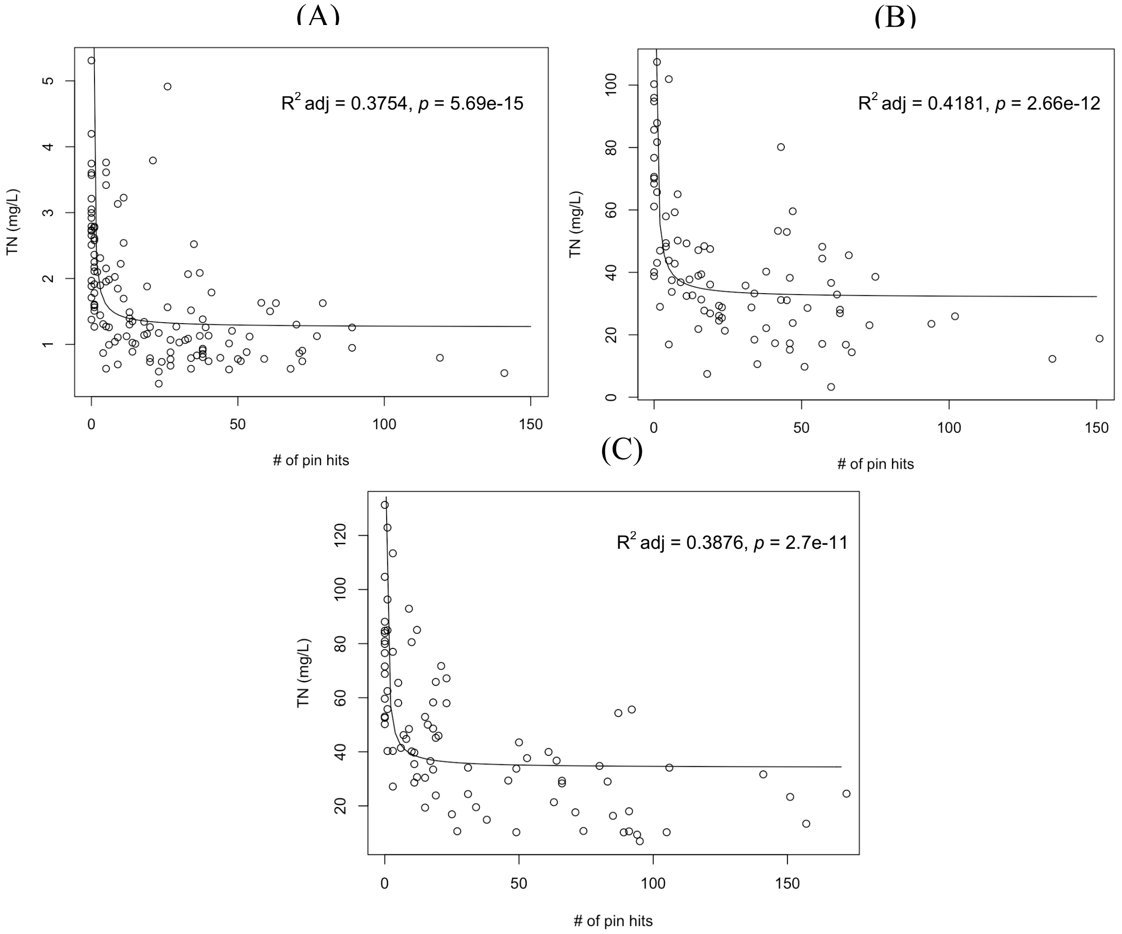

Figure 10. Linear regressions of runoff concentrations of total nitrogen (mg/L) compared to the number of pin hits for all treatments before fertilizer addition (n=130) and after fertilizer addition (n=91). (**A**) Pre-fertilizer ($F_{1,128}$ = 78.52, *p* = 5.7e-15); (**B**) Two weeks post-fertilizer ($F_{1,89}$ = 65.67, *p* = 2.66e-12); (**C**) Five weeks post-fertilizer ($F_{1,89}$ = 57.95 , *p* = 2.7e-111). Lines of best fit are represented by inverse regressions (y = 1/x) of transformed variables to maximize the R^2 –adjusted value. Plots are shown with raw data.

Furthermore, a one-way ANOVA was used to estimate the effect of vegetation treatment on runoff dissolved carbon concentrations (DOC). These results showed that vegetation treatment only had a significant effect on runoff DOC concentrations after fertilizer addition (two weeks: *p* = 0.01033; five weeks: *p* = 8.207e-06). Prior to adding the controlled release fertilizer, DOC concentrations ranged from a minimum of 4.8 mg/L to a maximum of 28 mg/L, with no significant variation among treatments ($F_{12,\,117}$ = 1.318, *p* = 0.2173). Following fertilizer addition, levels of DOC in the runoff increased, and showed significant variation among treatments. Samples from two weeks post-fertilizer show DOC concentrations ranged from 6.4 mg/L to a maximum outlier value of 187 mg/L (Figure 11A). Samples from five weeks post-fertilizer had much lower variation among treatments, with a range of 8.4 – 21.6 mg/L (Figure 11B). Contrary to runoff nitrogen concentrations, all three species mixtures were among the treatments with the highest DOC concentrations in the runoff.

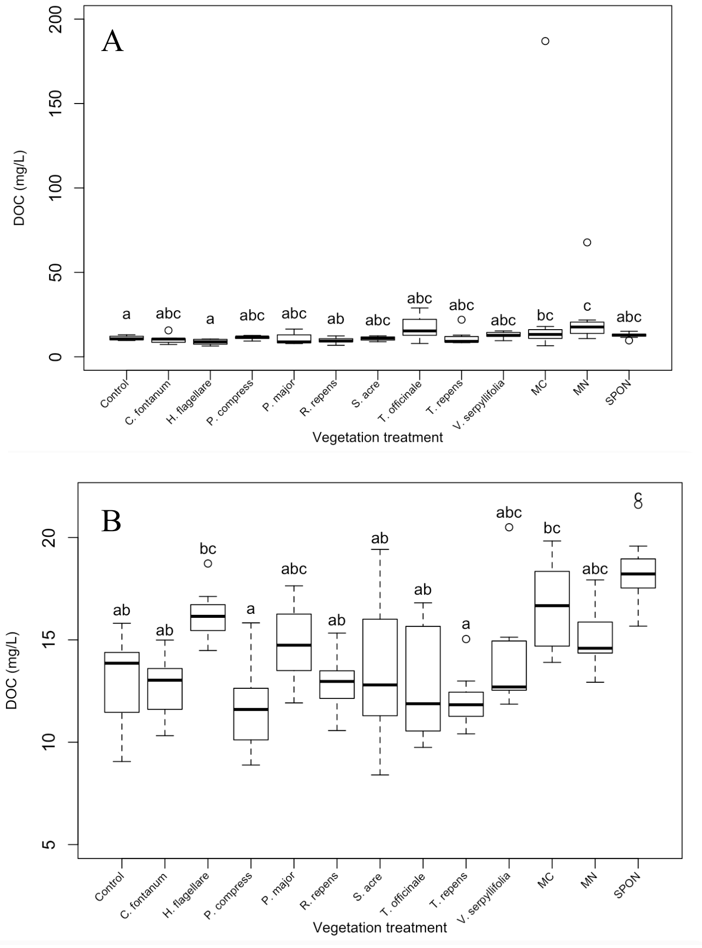

Figure 11. Box plots of runoff concentrations of dissolved organic carbon (DOC (mg/L)) sampled (**A**) two weeks after fertilizer addition ($F_{12,\ 78} = 2.4105$, $p = 0.01033$) and (**B**) five weeks after fertilizer addition ($F_{12,78} = 4.7785$, $p = 8.207\text{e-}06$). Monoculture treatments indicated by Latin names and species mixtures indicated by identifier codes: mixture with clover (MC); mixture without clover (MN); spontaneous colonization (SPON). Boxes without shared letters are significantly different ($p < 0.05$).

In addition, a one-way ANOVA was conducted to compare runoff nutrient concentrations within a species over time. These results showed that nutrient concentrations after fertilizer addition were significantly different than before fertilizer addition. Treatments producing runoff with the lowest TN and NO_3 concentrations include monocultures of *S. acre*, *T. officinale*, as well as all three species mixtures (Table 2, 3). Reduced runoff DOC concentrations were similar among all treatments (Table 4).

Table 2. Repeated measures analysis comparing changes of runoff nitrate (NO_3^-) concentrations within each treatment before fertilizer addition (pre-fert) and after fertilizer addition (post-fert). Values represent least squared means with standard error. Values without shared letters are significantly different within a vegetation type only ($p < 0.05$).

Vegetation Type	***NO_3^- (mg/L)***		
	Pre-Fert	Post-Fert 1	Post-Fert 2
Control	^{a} 2.75 ± 0.47	^{b} 70.15 ± 3.23	^{b} 63.59 ± 4.84
C. fontanum	^{a} 1.26 ± 0.223	^{b} 34.01 ± 2.86	^{c} 67.23 ± 8.26
H. flagellare	^{a} 0.027± 0.012	^{b} 22.7 ± 4.47	^{c} 34.54 ± 3.23
P. compressa	^{a} 0.079 ± 0.02	^{b} 32.25 ± 3.85	^{b} 37.34 ± 4.87
P. major	^{a} 1.11 ± 0.413	^{b} 33.29 ± 6.37	^{b} 43.45 ± 6.25
R. repens	^{a} 0.235± 0.09	^{b} 23.88 ± 2.33	^{c} 42.96 ± 4.28
S. acre	^{a} 0.095 ± 0.02	^{b} 18.57 ± 3.15	^{b} 14.89 ± 2.06
T. officinale	^{a} 0.428± 0.20	^{b} 22.39 ± 2.52	^{b} 22.6 ± 5.17
T. repens	^{a} 1.62 ± 0.37	^{b} 43.34 ± 3.12	^{c} 61.37 ± 4.01
V. serpyllifolia	^{a} 1.75 ± 0.27	^{b} 59.31 ± 6.88	^{c} 81.53 ± 10.83
Mixture with clover	^{a} 0.033 ± 0.008	^{b} 11.99 ± 3.27	^{b} 17.37 ± 3.74
Mixture no clover	^{a} 0.11 ± 0.03	^{b} 16.63 ± 2.5	^{b} 22.53 ± 4.16
Spontaneous	^{a} 0.054 ± 0.013	^{b} 28.04± 3.84	^{b} 23.4 ± 5.52

Table 3. Repeated measures analysis comparing changes of runoff total nitrogen concentrations within each treatment before fertilizer addition (pre-fert) and after fertilizer addition (post-fert). Values represent least squared means with standard error. Values without shared letters are significantly different within a vegetation type only (*p* < 0.05).

Vegetation Type	***Total Nitrogen (mg/L)***		
	Pre-fert	Post-Fert 1	Post-Fert 2
Control	^{a} 3.38 ± 0.417	^{b} 78.7 ± 5.62	^{b} 73.68 ± 6.59
C. fontanum	^{a} 1.91 ± 0.116	^{b} 56.36 ± 8.74	^{c} 84.25 ± 7.49
H. flagellare	^{a} 1.04 ± 0.148	^{b} 30.0 ± 5.15	^{b} 38.87 ± 3.89
P. compressa	^{a} 1.2 ± 0.173	^{b} 43.1 ± 6.85	^{b} 38.9 ± 5.11
P. major	^{a} 2.18 ± 0.359	^{b} 40.42 ± 6.68	^{b} 49.94 ± 8.10
R. repens	^{a} 1.06 ± 0.08	^{b} 29.2 ± 2.31	^{c} 43.96 ± 4.37
S. acre	^{a} 0.904 ± 0.116	^{b} 26.82 ± 4.66	^{b} 16.84 ± 2.35
T. officinale	^{a} 2.04 ± 0.443	^{b} 32.43 ± 3.88	^{b} 24.22 ± 5.22
T. repens	^{a} 2.13 ± 0.129	^{b} 44.05 ± 2.91	^{c} 64.88 ± 4.19
V. serpyllifolia	^{a} 2.31 ± 0.310	^{b} 77.02 ± 9.19	^{b} 87.82 ± 11.3
Mixture with clover	^{a} 1.0 ± 0.163	^{b} 17.0 ± 3.51	^{b} 20.4 ± 4.11
Mixture no clover	^{a} 1.01 ± 0.194	^{b} 22.28 ± 2.16	^{b} 23.25 ± 4.13
Spontaneous	^{a} 0.962 ± 0.09	^{b} 38.55 ± 5.67	^{b} 25.79± 5.50

Table 4. Repeated measures analysis comparing changes of runoff DOC concentrations within each treatment before fertilizer addition (pre-fert) and after fertilizer addition (post-fert). Values represent least squared means with standard error. Values without shared letters are significantly different within a vegetation type only (*p* < 0.05).

Vegetation Type	***DOC mg/L***		
	Pre-Fert	Post-Fert 1	Post-Fert 2
Control	^{a} 9.15 ± 0.974	^{ab} 11.11 ± 0.501	^{b} 12.92 ± 0.891
C. fontanum	^{a} 10.1 ± 1.185	^{a} 10.3±1.027	^{a} 12.7±0.617
H. flagellare	13.13± 1.922	8.75±0.666	16.24±0.528
P. compressa	^{a} 9.22 ± 0.930	^{a} 11.32±0.433	^{a} 11.69± 0.871
P. major	^{a} 10.7 ±1.781	^{a} 10.8±1.377	^{a} 14.8± 0.810
R. repens	^{a} 9.39 ± 0.794	^{ab} 9.66 ± 0.687	^{c} 12.88 ± 0.561
S. acre	^{a} 9.02 ± 1.12	^{ab} 10.82 ± 0.534	^{b} 13.60 ± 1.466
T. officinale	^{a} 11.3 ± 1.573	^{a} 17.4 ± 2.874	^{a} 13.0 ± 1.145
T. repens	^{a} 9.42± 1.176	^{a} 11.54 ± 1.842	^{a} 12.10 ± 0.583
V. serpyllifolia	^{a} 7.72 ± 0.668	^{b} 12.95 ± 0.761	^{b} 14.29 ± 1.134
Mixture with clover	^{a} 7.79 ± 0.568	^{a} 37.24 ± 24.99	^{a} 16.64 ± 0.882
Mixture no clover	7.93 ± 0.822	23.56 ± 7.521	15.13 ± 0.607
Spontaneous	^{a} 8.73 ± 1.054	^{b} 12.69 ± 0.647	^{c} 18.35 ± 0.713

3.2.2 Total runoff nutrient output

Similar to the process above used for runoff nutrient concentrations, a Shapiro-Wilks test was used to assess if the data were normally distributed. For cases of non-normally distributed data, a logarithmic function was used to transform the data to as close to normality as possible. A mixed model ANOVA was run using block as ‘random’ effect, and treatment as a fixed effect. Again, however, when compared against a ‘null’ factor, block effect was found to have low variance (0.027), indicating that the block likely does not have much of an effect on the data. This was adjusted by using a one-way ANOVA to determine the effect of vegetation treatment on the total amount of nutrients being exported out of the green roof system via runoff. This nutrient output value was calculated for every module of every treatment using the following equation:

Runoff volume of module (L) x $\boldsymbol{Nutrient\ concentration}\ (\frac{mg}{L})$ = total amt. of nutrients exported out of green roof system

These values were plotted, and a pairwise analysis was used to determine which treatment values were significantly different from one another. The results indicate that vegetation treatment had a significant effect on runoff total nitrogen (TN), as well as nitrate (NO_3), both prior to fertilizer addition (TN: $p = 2.034e\text{-}07$; NO_3: $p = 2.2e\text{-}16$), and following fertilizer addition (TN: $p < 3.9e\text{-}05$; NO_3: $p < 5.8e\text{-}07$). The amount of total nitrogen released from the green roof system was highly variable among treatments before and after fertilizer was added. There is however, a noticeable pattern of species mixtures exporting far less nutrients than monoculture treatments for samples taken five weeks after fertilizer was added (Figure 12C). These results are similar for nitrate, where species mixtures were among the treatments with the least amount of nitrate being exported out of the system via runoff, alongside monocultures of S. *acre* and T. *officinale* (Figure 13). In the case of DOC, treatment was found to have an effect on DOC output before and after fertilizer addition. However, although the ANOVA detected a significant effect for treatment of the samples taken two weeks after fertilizer addition (p = 0.0378), pairwise interactions determined no significant differences among treatment groups (Fig. 14).

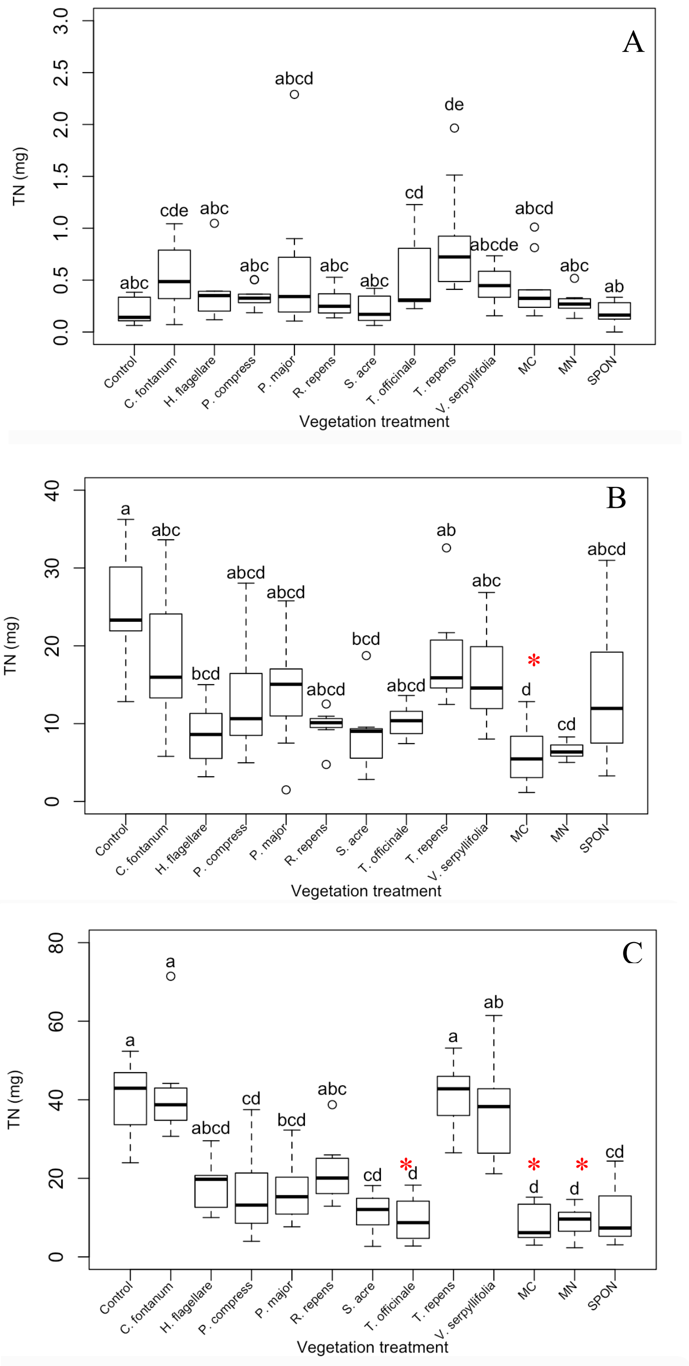

Figure 12. Box plot of total amount of TN (mg) exported from the green roof system (**A**) before fertilizer addition ($F_{12,117} = 5.5313$, $p = 2.034$ e-07),(**B**) two week after fertilizer addition ($F_{12,78} = 4.25$, $p = 3.86$e-05) and (**C**) five weeks after fertilizer addition ($F_{12,78} = 10.57$, $p = 4.65$ e-12). Monoculture treatments are indicated by Latin names and species mixtures indicated by identifier codes: mixture with clover (MC); mixture without clover (MN); spontaneous colonization (SPON). Boxes with an asterisk* indicate the lowest amount of total nitrogen exported out. Boxes without shared letters are significantly different ($p < 0.05$).

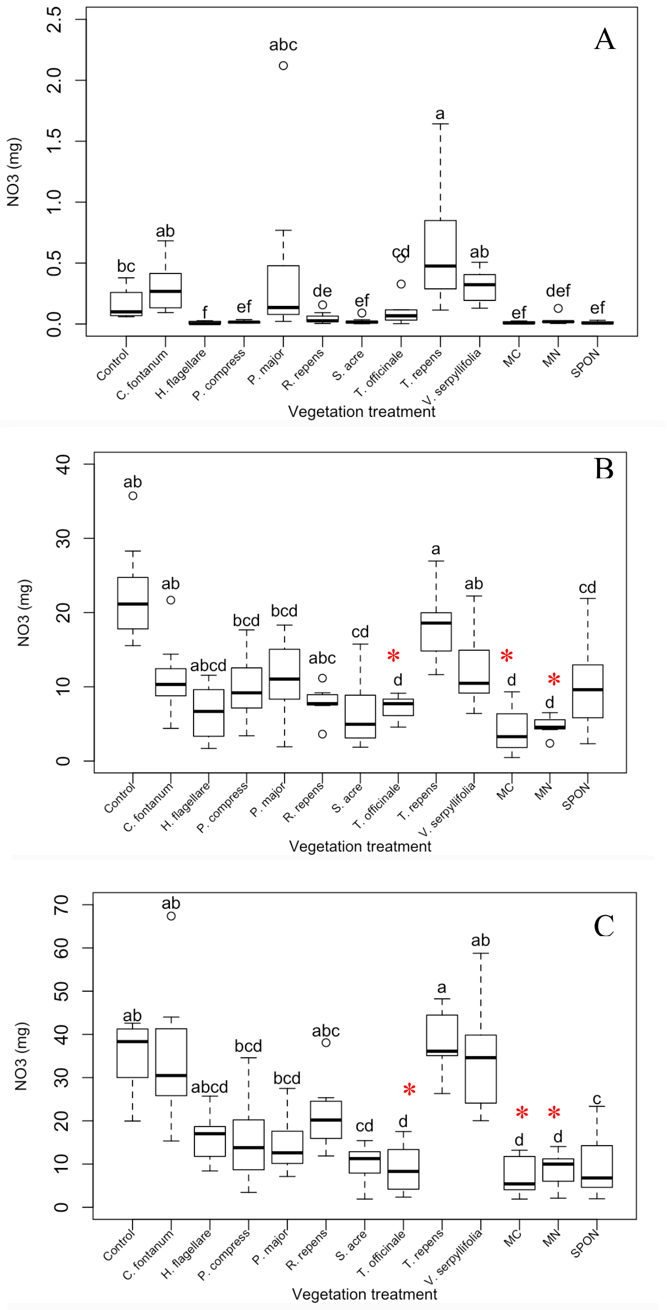

Figure 13. Box plots of total amount of NO_3^- (mg) exported from the green roof system (**A**) before fertilizer addition ($F_{12,117} = 26.46$, $p = 4.65$ e-12), (**B**) two weeks after fertilizer addition ($F_{12, 78} = 9.1$, $p = 1.2e\text{-}10$) and (**C**) five weeks after fertilizer addition ($F_{12, 78} = 9.05$, $p = 1.34e\text{-}10$). Monoculture treatments are indicated by Latin names and species mixtures indicated by identifier codes: mixture with clover (MC); mixture without clover (MN); spontaneous colonization (SPON). Boxes with an asterisk* indicate the lowest amount NO_3 exported out. Boxes without shared letters are significantly different ($p < 0.05$).

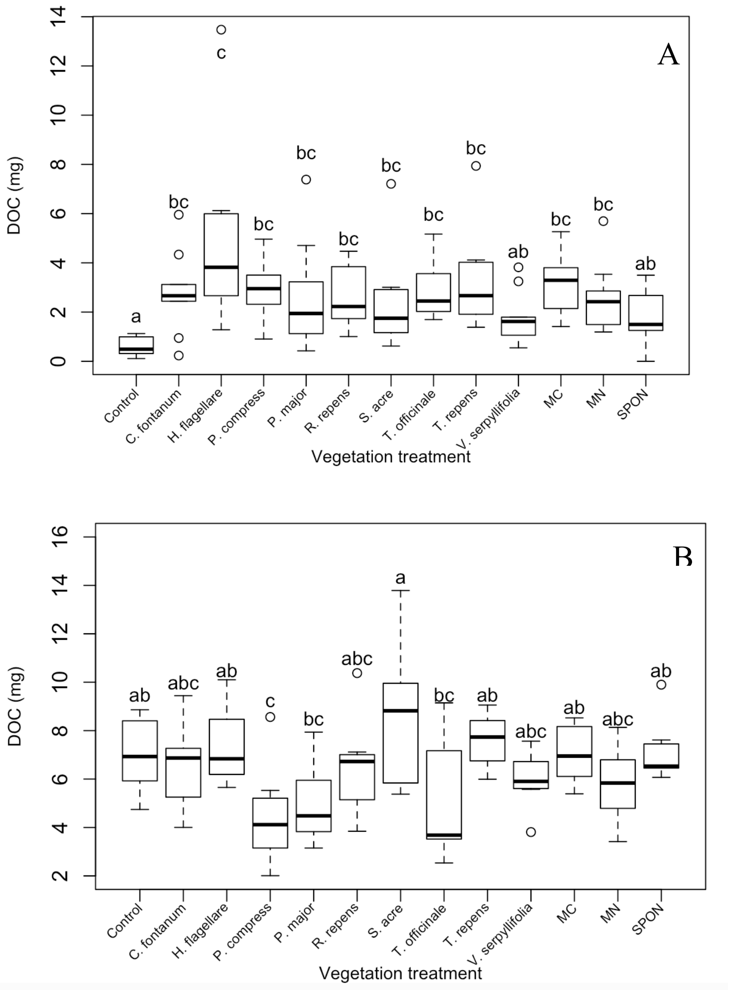

Figure 14. Box plots of total amount of DOC (mg) exported to the environment (**A**) before fertilizer addition ($F_{12,117} = 5.2438$, $p = 5.25e\text{-}07$) and (**B**) five weeks after fertilizer addition ($F_{12,117} = 3.06$, $p = 0.00142$). Monoculture treatments are indicated by Latin names and species mixtures indicated by identifier codes: mixture with clover (MC); mixture without clover (MN); spontaneous colonization (SPON). Boxes without shared letters are significantly different ($p < 0.05$).

3.3 Growing media nutrient concentrations

To determine if the block had any effect on soil nutrient data, two separate mixed model ANOVA's were run; one using 'block' as random effect, and the other using a 'null' factor as random effect. Comparison of these two models showed that there was no improvement in fit when the random effect was included. Instead, a one-way ANOVA was used to estimate the effect of vegetation treatment on soil nutrient concentrations.

Results showed that vegetation treatment had a significant effect on soil nutrient concentrations for both un-fertilized and fertilized samples. Soil NO_3^- concentrations of un-fertilized samples produced similar patterns to those seen in the runoff, where species mixtures were found to have the lowest concentrations of soil nitrate, whereas the control treatment, plus a few other monocultures were found to have the highest concentrations (Figure 14C). This result differed slightly for samples *with* fertilizer added, where the spontaneously colonized treatment (SPON) was the only mixture to produce values significantly lower than all other treatments (Figure 15D).

For phosphorus (P_2O_5), monocultures of S. *acre* were found to have the lowest concentrations, while control treatments had the highest concentrations for both fertilized and un-fertilized samples. Treatments produced a wide spectrum of P_2O_5 concentrations, ranging from a minimum of 232 ppm in unfertilized samples, to a maximum 1946 ppm in fertilized samples (Figure 15A, B).

Some patterns were similar for potassium (K_2O) concentrations. A wide range of K_2O concentrations were found, with 50 ppm being the minimum in un-fertilized samples, and 1254 ppm being the maximum in fertilized samples. For un-fertilized samples, control treatments had the lowest concentrations, and the mixture without clover (MN) had the highest concentrations (Figure 15E). Contrastingly, an opposite result was seen in the fertilized modules, where the spontaneous mixture (SPON) was found with the lowest concentrations, and control treatments (along with a few other monocultures) were found with the highest concentrations (Figure 15F).

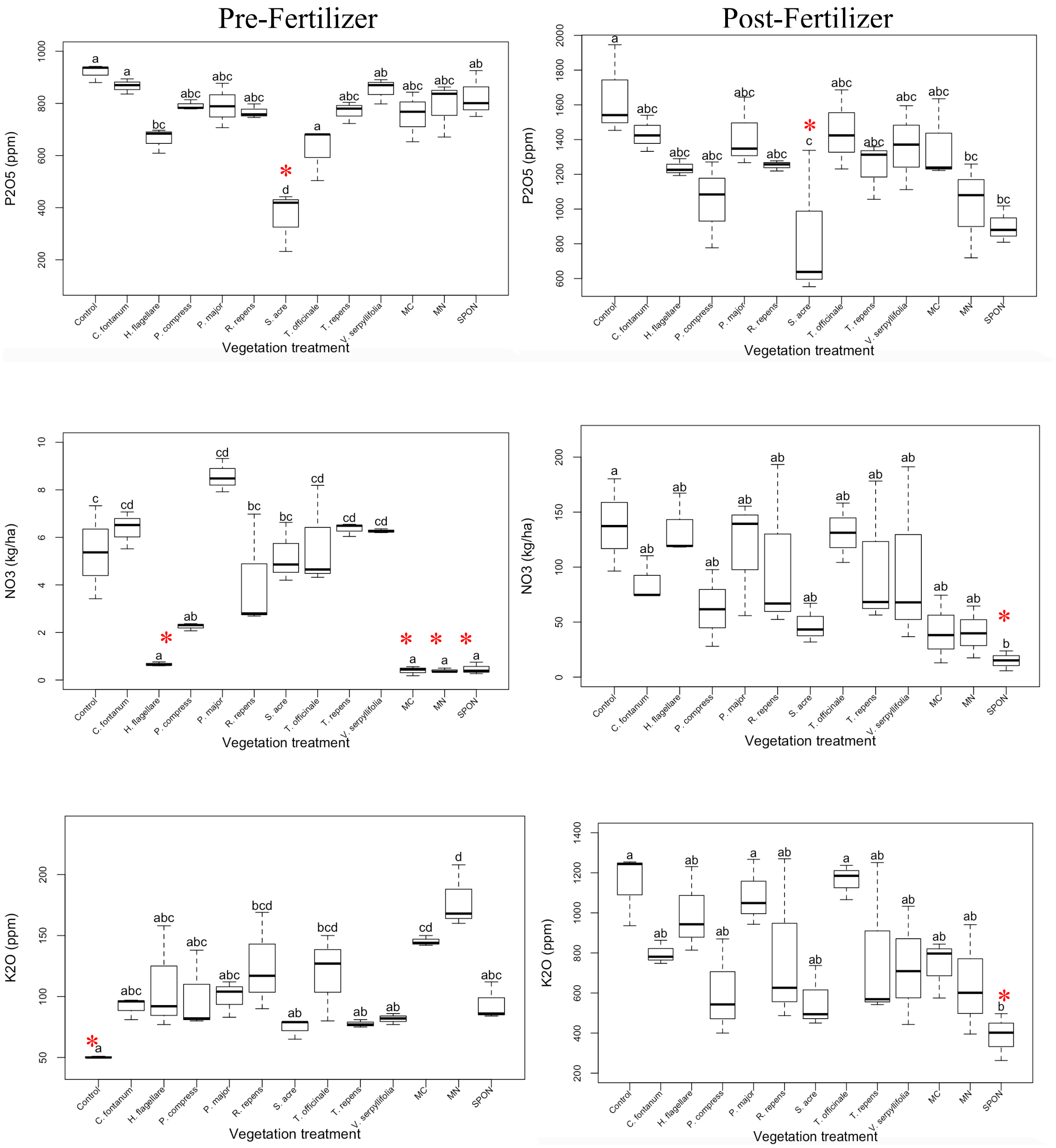

Figure 15. Box plots of soil nutrient concentrations for all vegetation treatments. (**A**) P_2O_5 (ppm) for un-fertilized modules, (**B**) P_2O_5 (ppm) for fertilized modules. (**C**) NO_3 (kg/ha) for un-fertilized modules; (**D**) NO_3 (kg/ha) of fertilized modules. (**E**) K_2O (ppm) for un-fertilized modules. (**F**) K_2O (ppm) of fertilized modules. Monoculture treatments indicated by Latin names, species mixtures indicated by identifier codes: mixture with clover (MC); mixture without clover (MN); spontaneous colonization (SPON). Boxes with an asterisk* indicate significantly lowest nutrient concentrations. Boxes without shared letters are significantly different ($p < 0.05$).

4. DISCUSSION

4.1 Canopy density

Results indicated that fertilizer had an effect on plant growth, causing some treatments to exhibit a significant increase in aboveground biomass when measured over a period of time. This result, however, was found only for a subset of vegetation types such as species mixtures, and monocultures of *P. compressa*, *P. major*, *R. repens*, and *S. acre*. This variation in growth among different vegetation types suggests that some species, or mixtures of species, function better at taking up available soil nutrients than others. As plants differ in their nutrient requirements, it is also possible that those species/mixtures that exhibited significant growth had a greater affinity for nutrient uptake, at least for the types of nutrients made available by the slow-release fertilizer, hence their increased canopy density over time. For monocultures that did not have a significant increase in growth, it is possible these species do not grow as well as others in isolation. For example, white clover (*T. repens*) is commonly found in lawns and pastures across several continents, but rarely grows as a monoculture. Greater plant diversity, and incorporating a mix of vegetation types, has been shown to enhance provisioning ecosystem services, particularly in a green roof environment (Lundholm *et al*. 2010). For monocultures that showed a decrease in growth between post-fert 1 and post-fert 2, it is possible the sudden change in aboveground biomass reduced these species' resilience. Given this green roof system had no supplemental irrigation, it is possible these species were not getting enough water during periods of 'drought' to sustain their needs from increased biomass production (Appleby-Jones *et al.* 2017). While there is sufficient research studying fertilization of *Sedum*-exclusive green roofs, there is less exploration of fertilization of mixed vegetation, such as the mixtures used in this study. Based on results of nutrient concentrations of the growing media pre-fertilizer application, it seems as though aboveground biomass of this system may limited by nutrient availability in the soil. As many ecosystem services are related to biomass, further exploration of the effects of fertilizer application to this green roof system is encouraged.

4.2 Runoff volume

The volume of runoff produced from each module can be a rough indication of that species' or mixture's ability to influence water retention in a green roof system, with lower runoff associated with greater benefits (as there are environmental and economic costs associated with runoff from buildings). Although I expected to see the highest runoff volumes in modules with bare substrate, results prior to fertilizer addition showed that control treatments (bare substrate) had consistently *low* volumes of runoff compared to all other vegetation treatments. While it is well known that vegetated roofs reduce runoff through storm water retention via the greater water-holding capacity of soils or growing media compared with conventional roofs, it is not clear how much of the water retained is attributable to substrate retention capabilities, or is actually taken up by the plants and returned to the atmosphere through transpiration. A study by VanWoert *et al*. (2005) found that, although significantly different from gravel roofs, vegetated roofs and non-vegetated roofs (growing media only), were not statistically different in retention when categorized by rainfall events. The study suggested that physical properties of the growing media, as well as the presence of a water retention mat, are main components influencing water retention in a green roof system. Other studies looking at storm water retention in green roof systems further emphasize that many factors influence a roof's ability to retain water (Sims et al. 2016; Zhang et al. 2018).

A study by Zhang et al. (2018) describes how plant root systems are likely a major factor influencing water retention. Root systems can act as conduits by creating preferential flow pathways in the substrate by increasing water flux downward through macropores and channel networks (Johnson et al. 2016). This tells us root systems may play a significant role reducing water retention in extensive green roof systems (such as the one in this study) due to shallower substrate depths.

There is also the possibility, however, for environmental factors to be at play. Climate conditions likely had a strong influence during the early growing season when considering the cooler air temperatures found in this geographic region during the spring. Air temperature records for Halifax, NS show a mean temperature of 0°C and 4°C for the months of March and April 2018, respectively (CustomWeather ©2019). These cooler temperatures may have caused vegetation to reduce or prevent evaporation, when comparing runoff values to the control groups. As well, temperature could have affected transpiration demands by the plants, as less water would be required with cooler substrate temperatures.

Additionally, given that there was no supplemental irrigation of the green roof system prior to, or during the data collection period, lack of precipitation could have resulted in dry substrate. Modules with little to no vegetation (such as the control treatment) are more likely to dry out, due to lack of vegetation cover cooling the substrate and retaining moisture. Once modules were watered during data collection periods, those that had less substrate moisture were more likely to retain water, and thus produce a lower volume of runoff. This effect is seen particularly in cooler, wetter climates where plant water demands are relatively low, and in turn may reduce the efficacy of green roofs contribution to storm water management in these settings.

Conversely, volumes of runoff for the unvegetated (control) treatments were found to increase in the later sampling periods. Again, these results are likely influenced by climate conditions, specifically air temperatures around the sampling period. As opposed to the pre-fertilizer data, which was collected following the cooler spring season, post-fertilizer data was collected late in the growing season, following weeks of warmer weather. For the second post-fertilizer sampling period specifically, temperature records showed mean temperatures of 32°C and 21°C for the months of July and August, respectively (CustomWeather ©2019). Here, the hotter temperatures are likely driving higher transpiration rates by the vegetation. In these instances, plant roots remove water from the substrate more effectively than evaporation alone from the surface.

These findings are also consistent with results from linear regression analyses that found a very weak positive correlation present between aboveground biomass and runoff volume. These analyses are in also support of previous research that found biomass to be an ineffective indicator of water retention (Lundholm *et al.* 2010; Johnson *et al.* 2016; Zhang *et al.* 2018).

It may be important to note that our canopy density index, however, does not take belowground biomass into account. As mentioned previously, root systems play a major role in both uptake and water movement through the substrate, making belowground biomass and root traits important factors to consider in future studies of green roof runoff volume.

While most monocultures and species mixtures had similar averages for runoff volume, monocultures of S. *acre* and T. *repens* were similar to the control treatments in having significantly higher volumes of runoff. Seeing as T. *repens* had very little above ground biomass during this sampling period, it is likely that this treatment followed similar patterns to that with bare substrate, where evaporation from the soil surface would provide the main mechanism controlling the amount of water retention. As for S. *acre*, past studies have shown this vegetation type to be relatively ineffective for reducing runoff in green roof systems, particularly when compared to grasses and forbs (Dunnett *et al.* 2008). As well, as a succulent species, *S. acre* has low transpiration demands relative to other vegetation types, and thus does not retain as much water. This species also grows in a thick, mat-like fashion, and likely prevents evaporation from the substrate surface. The combination of these factors support the much higher values seen in *S. acre* runoff volumes.

4.2.1 Runoff nutrient concentrations

When comparing nutrient concentrations prior to and following fertilizer addition, there is a clear indication that adding fertilizer results in increased nutrient concentrations in the runoff. There are, however, noticeable patterns in which treatments are producing the lower or higher concentrations. These patterns indicate that the treatments producing low nitrogen and nitrate concentrations before fertilizer are the same treatments producing low concentrations after fertilizer addition, sampled at both two weeks and five weeks post-fertilizer. These results suggest that the effects of different vegetation types are observable as a short-term response to fertilization, but also likely as long-term patterns in nutrient uptake.

Overall, treatments that produced the lowest concentrations of nitrate and total nitrogen both prior to and following fertilizer addition were the three species mixtures, as well as monocultures of *S. acre*, *H. flagellare* and *T. officinale*. These treatments generally had more aboveground living biomass than other treatments, suggesting that biomass is a driving factor behind nutrient uptake. This suggestion is further supported by the treatments that produced the highest nutrient concentrations. Such treatments were the control, and monocultures of *C. fontanum*, *T. repens* and *V. serpyllifolia*, which were generally found to have little to no aboveground biomass. Linear regression analyses determined that there is a relationship between biomass and runoff nutrient concentrations up to a certain point. From the results of these analyses, an inverse correlation was found between runoff concentrations and number of pin hits. These figures indicate that there is a ‘threshold’ below which increasing biomass strongly decreases the amount of nutrients being leached into the runoff. This ‘threshold’ was around ~50 pin hits, where nutrient concentrations begin to level off as the number of pin hits increases.

It is possible that increased biomass may have more of an effect than we’re seeing here, as our estimation of canopy density is not a true reflection of the quantity of living plant biomass functioning in the green roof system. Obtaining a more accurate value for the amount of total living biomass in each module (above and below ground), has the potential to more accurately demonstrate the strength of the relationship between biomass and runoff nutrient concentrations. Overall, these results indicate that selection of vegetation type, and in particular higher biomass species or species mixtures, can help mitigate increased nutrient loading in the runoff of vegetated roofs.

Looking at dissolved organic carbon, results indicate there are different factors influencing DOC concentrations in the runoff. Here, vegetation type only had a statistically significant effect after fertilizer addition. Interestingly, species mixtures were found to have the highest runoff concentrations of DOC, compared to nitrogen where they had the lowest concentrations. Although still unclear, this result could likely in part be attributable to biomass as well. While there is still much unknown regarding the physiological role of root exudates, studies have shown root cells and exudates to function as a major input pathway of organic carbon into the soil (Rasse *et al*. 2005; Jones *et al.* 2009). Therefore, increased biomass should result in an increased production of root exudates by the plants, which could also help explain the elevated DOC concentrations in the higher biomass treatments, which are in turn leaving via runoff.

While DOC is an important part of carbon cycling, and is beneficial to aquatic organisms as a food source (Song *et al*. 2018; Bjork and Gilek, 1996), high concentrations of DOC can be harmful from environmental, ecological, and anthropogenic standpoints. In regards to runoff in urban ecosystems and water treatment, elevated DOC concentrations cause complications. Dissolved organic carbon reacts with the chlorination process in the treatment of drinking water, producing harmful haloform compounds known as trihalomethanes (Moore, 1998; Health Canada, 2006). Elevated levels of DOC in watersheds and waterways may ultimately lead to increased water treatment, which has economic costs.

When looking within a vegetation type only, higher runoff nutrient concentrations observed in a later sample (five weeks post-fertilizer) compared to an earlier sample (two weeks post-fertilizer), could be attributed to the behaviour of the controlled-release fertilizer. This fertilizer does not immediately release all the nutrients into the soil when applied, but does so steadily over a longer period of time. The result mentioned above was consistent for low biomass treatments such as *C. fontanum*, *H. flagellare*, *R. repens*, and *T. repens*. As less biomass typically results in less nutrient uptake, more nutrients are accumulated in the substrate and are thus leached out in in the runoff. Conversely, treatments that had lower nutrient concentrations in the later sample (five weeks post-fertilizer) rather than earlier samples (two weeks post-fertilizer) could be attributed to increased plant growth, which in turn would increase nutrient uptake, and leave less available in the substrate to leach out in runoff. This result was typically seen in higher biomass treatments, such as *P. compressa*, *P. major, S. acre*, *T. officinale,* and species mixtures.

In both monocultures and succulent mixtures, S. *acre* has repeatedly demonstrated its high levels of efficacy in provisioning many green roof ecosystem services. This study is no exception; runoff from S. *acre* had the lowest nutrient concentrations of all monocultures, while consistently producing high biomass, which further indicates this species thrives in this type of environment, especially when compared to the other monocultures. Something worth noting, however, is that all species mixture treatments produced runoff quality similar to S. *acre* in terms of low nutrient concentrations, while also producing high aboveground biomass. Again, this is not surprising given many studies have found mixtures to out-perform monocultures, a term known as mixture advantage or overyielding (Lundholm, 2015). This result does spark interest, however, regarding the spontaneous colonization treatments, in which no plants were planted at the beginning of the experiment at all. The treatment was able to colonize itself naturally, yet still produced results that are, on some levels, just as good as a ‘top’ green roof vegetation type (i.e. *Sedum* spp.). This information supplies evidence that importing non-native species such as *Sedum* may not be necessary to achieve high levels of environmental performance. Further investigation is encouraged into the provisioning of other green roof ecosystem services, such as substrate cooling and water retention attributed to more biomass from fertilizer application. In addition, further research should be conducted into whether increased nutrient load in the runoff after fertilizer application is partially mitigated by the increase in other services.

4.2.2. Implications of precipitation rate

When looking at results of nutrient concentrations in the runoff, it is important to note the rate at which the water (“precipitation”) was applied to each module. Due to time and resource restrictions, the rate of the ‘simulated’ rain event used in this experiment was chosen to ensure all modules underwent data collection on the same day. Based on the calculations below, the rate of simulated precipitation for each module equates to 0.5 mm rain/sec, or 30 mm rain/ min:

$$0.1296 \text{ m}^2 \text{ x } \frac{100\ (dm2)}{1\ (\text{m}2)} = 12.96 \text{ dm}^2$$

$$\frac{2\ d\text{m}2\ H2O}{12.96\ d\text{m}2} = 0.154 \text{ dm} = 15.4 \text{ mm } H_2O \text{ / 30 sec}$$

$$= \mathbf{0.51\ mm}\ \text{‘rain’ / sec}$$

Knowing this information, it should be noted that the runoff concentrations of nutrients in this study may represent only a fraction of natural rain events that occur at that intensity in this local climate, and in turn these values could be an over-estimation. However, these values are not inconsistent with the amount of water that could be applied to a green roof via a sprinkler system or hand-watering with a hose. Overall, the high rate of precipitation used in this experiment could very well have an effect on the amount of nutrients being leached out of green roof system at a given time.

There are however, studies that have found a "first flush" effect when looking at runoff nutrient concentrations from vegetated roofs. The "first flush" effect describes an event where initial runoff from storm water contains higher concentrations of nutrients than later runoff, despite a larger volume of water applied later on (Bertrand-Krajewski *et al*. 1998). A study by Berndtsson et al. (2008) examined runoff nutrient concentrations from a variety of vegetated and non-vegetated roofs sampled at different times during a simulated rain event. The study found that nutrients (including nitrogen and nitrate) had proportionally higher concentrations in the runoff earlier rather than later on, which was indicative of a first flush effect. These results suggest that the rate of precipitation applied to a green roof system may not have as much of an effect on newly constructed green roofs, or vegetated roofs that are heavily fertilized, where a first flush effect is more likely to occur, regardless. This may lead to recommendations regarding fertilizer application for newly constructed green roofs, and overall fertilizer application rates and frequencies.

4.2.3 Total runoff nutrient output

Overall, the total amount of nutrients being exported out of the green roof system via runoff was highly variable among different vegetation types. This high level of variability is likely the result of runoff volume being much less correlated with biomass compared to nutrient concentrations. There are, however, some similar patterns in total nitrogen and nitrate output as seen in the runoff concentrations. Species mixtures still had consistently lower values of total nutrient output than most monoculture treatments. Moreover, *T. repens* (white clover) was among the highest values for both total nitrogen and nitrate. While this may in part be attributable to the nitrogen-fixation capabilities within the roots of this species (Carlsson and Huss-Danell, 2003), it is more likely an effect of low biomass, as *T. repens* did not differ significantly from the control treatment in most cases, and pre-fertilization substrate nitrate levels were not higher in *T. repens* than controls or any other vegetated treatment. As mentioned previously, low biomass results in less nutrient uptake, and this is a likely explanation for the higher amounts of nitrogen and nitrate being exported by these treatments.

For dissolved carbon, statistical tests detected no significant differences among treatments except for the control in the pre-fertilized sample, which was significantly lower, and *S. acre* in the post-fertilized sample which was significantly higher. These result, again, are likely attributable to biomass for the same reasons discussed in the runoff concentrations.

4.3 Soil nutrient concentrations

Given that the green roof system used in this study is one that has been established for five years, and no growing media has been added since it was set up, samples of the un-fertilized growing media can lend insight into nutrient dynamics between different vegetation types over a longer period of time. Growing media sampled from species mixtures were found to have low nitrate concentrations, similar to the pattern found in the runoff results. As these were high biomass treatments that produced low nutrient levels in both the runoff and substrate, it further supports the idea that biomass is a driving force behind nutrient uptake over the long-term.

For nutrients that are not as readily taken up from the soil, such as phosphorus, we see a shift in the dynamics compared to that of nitrate. Phosphorus (P) levels of the growing media were found to be quite high, even before the addition of fertilizer. This result is relatively unsurprising considering the more complex, chemical and physical behaviour of P within the substrate. The phyto-availability of P depends on its ability to undergo adsorption or desorption, as well as the buffering capacity of the substrate (Wang and Liang, 2014). This factor makes it more difficult to predict if P would likely appear in the runoff, and at what concentrations. That being said, a study by Berndtsson et al. (2005) found phosphorus present in the runoff sampled from multiple vegetated roofs constructed 3-4 years prior to the study.

An interesting result to note, are the much lower values of P for monocultures of *S. acre*, both pre and post-fertilizer addition. Although the reason is still unclear, these results suggest *S. acre* is perhaps more effective at using available P in the growing media, or has greater physiological requirements for P than other species, and this is consistent with previous studies in a similar green roof system (Lundholm 2015). As a result, this species could likely reduce concentrations of P leached from the growing media. In any case, future analysis of P concentrations in runoff is highly encouraged.

Lastly, results show that potassium (K) levels of the growing media were noticeably lower pre-fertilizer addition than post-fertilizer addition. Unlike nitrate and P, K is not a natural component of organic soil, and therefore elevated levels of K would likely come directly from a fertilizer, such as the one applied in this study. Given that K leaching is strongly affected by substrate texture and K availability (Rosolem *et al*. 2010), these results make it difficult to predict trends in runoff K concentrations. As with P, further analysis of this nutrient in the runoff of a vegetated roof is encouraged to better understand different nutrient dynamics among vegetation types.

4.4 Limitations of the study

As this study took place on a roof in a natural environment, there were a number of external factors that could not be controlled in the period of time the experiment took place. For example, factors such as temperature, the amount of sunlight, and the number of natural precipitation events that occurred in between sampling periods were all uncontrolled environmental variables that could have influenced the data.

Additionally, due to time and resource restraints for this particular study, the sample size of precipitation events used for data collection was small (only three "rainfall" events were actually sampled). Furthermore, these events occurred over the course of only a few months, and results therefore only represent a snapshot in time. A study by Buffam et al. (2016) found large temporal variation in green roof water quality when sampled over a period of two years. These results emphasize the importance for long-term studies on runoff quality from vegetated roofs as a means of better understanding the fluctuation and variation seen in established green roof ecosystems.

5. CONCLUSION & FUTURE WORK

In summary, fertilizer application significantly increased aboveground biomass for over half the vegetation treatments in this study. These higher biomass species and species mixtures also resulted in lower concentrations of both total nitrogen and nitrate in the runoff, as well as the growing media. Overall, results from this study suggest that biomass, as well as choice of vegetation, is a key factor influencing nutrient dynamics within green roof systems. Therefore, selection of high biomass species, or mixtures of species can help produce a better runoff quality through mitigation of nutrient loading in the runoff.

Findings from this study also supply foundational knowledge regarding the effects of a controlled-release fertilizer applied to an extensive vegetated roof. Further research studying the effects of fertilizer application on runoff quality from roofs with mixed vegetation, or spontaneously colonized vegetation, will contribute a higher level of knowledge to runoff quality as a growing subfield of green roof research. Additionally, research into application rates, frequencies and formulations of fertilizers is encouraged to optimize the services provided by green roofs.

6. LITERATURE CITED

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