Fine-scale substrate heterogeneity in green roof plant communities: The constraint of size

Amiel Vasl1 | Bracha Y. Schindler1 | Gyongyver J. Kadas1,2 | Leon Blaustein1

1Kadas Green Roofs Ecology Research Center, Institute of Evolution and Department of Evolutionary and Environmental Biology, Faculty of Natural Sciences, University of Haifa, Haifa, Israel
2Environmental Research Group, Sustainability Research Institute, University of East London, London, UK

Correspondence
Amiel Vasl, Kadas Green Roofs Ecology Research Center, Institute of Evolution and Department of Evolutionary and Environmental Biology, Faculty of Natural Sciences, University of Haifa, Haifa, Israel. Email: amielvasl@gmail.com

Funding information
Kadas Family; Haifa University

Abstract
Heterogeneity–diversity relationship (HDR) is commonly shown to be positive in accordance with classic niche processes. However, recent soil-based studies have often found neutral and even negative HDRs. Some of the suggested reasons for this discrepancy include the lack of resemblance between manipulated substrate and natural settings, the treated areas not being large enough to contain species’ root span, and finally limited-sized plots may not sustain focal species’ populations over time. Vegetated green roofs are a growing phenomenon in many cities that could be an ideal testing ground for this problem. Recent studies have focused on the ability of these roofs to sustain stable and diverse plant communities and substrate heterogeneity that would increase niches on the roof has been proposed as a method to attain this goal. We constructed an experimental design using green roof experimental modules (4 m²) where we manipulated mineral and organic substrate component heterogeneity in different subplots (0.25 m²) within the experimental module while maintaining the total sum of mineral and organic components. A local annual plant community was seeded in the modules and monitored over three growing seasons. We found that plant diversity and biomass were not affected by experimentally created substrate heterogeneity. In addition, we found that different treatments, as well as specific subplot substrates, had an effect on plant community assemblages during the first year but not during the second and third years. Substrate heterogeneity levels were mostly unchanged over time. The inability to retain plant community composition over the years despite the maintenance of substrate differences supports the hypothesis that maintenance of diversity is constrained at these spatial scales by unfavorable dispersal and increased stochastic events as opposed to predictions of classic niche processes.

KEYWORDS
neutral theory, niche theory, plant community assemblage, plant–soil interactions

1 | INTRODUCTION

One of the longest standing challenges in the field of ecology is explaining the mechanisms that sustain species richness over time and space. Spatial heterogeneity of resources and environmental conditions was suggested to increase niches which would, in turn, support the maintenance of a variety of species (Chesson, 2000; MacArthur & Levins, 1964). Plants were previously used to show...
that the maintenance of a diverse plant community is a direct result of fine-scale heterogeneity where different plant species are supported by different patches (Whittaker, 1965).

Accumulating evidence for contradicting hypotheses resulted in the publication of the neutral theory (Hubbell, 2001) that successfully predicted observed patterns while completely ignoring resource heterogeneity. Although these contradicting theories were generally reconciled into a “niches–neutral continuum” (Leibold & McPeek, 2006; Matthews & Whittaker, 2014), the underlying insight was that the seemingly obvious heterogeneity–diversity relationship (i.e., HDR) was no longer indisputable.

This shaking of the niche theory may have given rise to the emergence of several studies that have challenged the generality of positive HDR especially in soil heterogeneity and even suggested negative HDRs (Gazol et al., 2013; Lundholm, 2009; Tamme, Hiesalu, Laanisto, Szava-Kovats, & Pärtel, 2010). Experimental studies that put this theory to test only rarely found a positive HDR for soil heterogeneity (Williams & Houseman, 2014). A large-scale meta-analysis was performed (Stein, Gerstner, & Kreft, 2014) and showed a significantly positive HDR effect across taxa, biomes, and spatial scales which could have potentially refuted the negative HDR studies. However, the meta-analysis only included large-scale (>10 km²) observational studies while the contradictory results were attained in experimentally manipulated fine-scale studies.

Some have tried linking this discrepancy to the effect of patch size. A meta-analysis performed on soil manipulations studies (Tamme et al., 2010) claimed that experimental studies’ negative HDR was limited by fine-scale patch size where fine-scaled heterogeneity supported lower diversity. This is also supported by the strong positive effect of patch size found in the meta-analysis performed on observational soil studies (Tamme et al., 2010) and, in general, HDR studies (Stein et al., 2014). Since experimental studies are inherently limited in their dimensions, it can be suggested that the manipulated patch size is innately limited by experimental dimensions due to physical restrictions which may explain the scarcity of positive HDR effects.

The attempt to reconcile negative HDRs in experimentally manipulated studies with the general positive perceived trend received three different potential hypotheses that were suggested or tested: lack of realism is embedded in the method of man-made heterogeneity, patch size effect on individuals, and patch size effect on populations.

Hypothesis 1: Realism in the method of creation of heterogeneity.

It has been claimed that a lack of realism is inherent in most methods of heterogeneity manipulation, especially with nutrient manipulations (Williams & Houseman, 2014). The manipulated substrates may not mimic natural soil, and nutrients that are artificially added may disturb plant-soil microbe interactions or, in certain cases where highly mobile forms of nitrogen are used, give preference to nitrophilic species that are able to capitalize on the resources more easily, which masks the heterogeneity effect.

Hypothesis 2: Patch size has effect on individuals.

Treated patch size within experimental modules has been targeted for some time as a potential challenge in studies of this kind; treated areas that are smaller than the root span of certain species are functionally invisible to those species (Hutchings, John, & Wijesinghe, 2003). However, when all species have similar root spans that are larger than treated patches, the heterogeneity effect is predicted to be neutral. When some species’ root spans are smaller and some are larger than patch size, species with larger root spans have a foraging advantage over species with smaller root spans and increase their fitness which could potentially reduce diversity (Rajaniemi, 2011; Tamme, Gazol, Price, Hiesalu, & Pärtel, 2016).

Hypothesis 3: Patch size has effect on populations.

Theoretical models designed to improve our understanding of community dynamics within heterogeneous surroundings found support for the negative HDR (Kadmon & Allouche, 2007; Palmer, 1992; Smith & Lundholm, 2012). This is explained by the increased stochasticity caused by habitat heterogeneity which affects plant populations. Reducing the absolute patch area results in smaller populations in each of the patches which in turn increase the chances of stochastic events occurring within them. An important role was also assigned to dispersal mechanisms—smaller patches would increase the percentage of propagules dispersed from the patches into unsuitable habitats due to the fact that patch perimeter would be closer to the plant and would also reduce the incoming propagules from the regional species pool (Kadmon & Allouche, 2007). At reduced patch sizes, increased heterogeneity has a better chance of causing a negative HDR.

In this experiment, we wish to put two of these hypotheses (2 and 3) to test. The construction of large experimental modules (=units) with large enough subplots (=patches) to sustain distinct plant populations and communities and manipulating mineral substrate components alongside observation and sampling over several years will allow us to examine the first and third hypotheses more closely. HDR as well as comparing community compositions between treated modules and subplots within modules could potentially shed light on the processes taking place. While substrate heterogeneity was predicted to increase plant diversity, we did not expect that it would increase plant biomass.

The increasingly common green roof studies may serve as an ideal testing ground for questions of this type. Green roofs are a widespread urban phenomenon where a vegetative layer is placed on roofs. The majority of green roofs are lightweight and often planted with a small array of plant species that entail minimal maintenance (Oberndorfer et al., 2007). While green roofs were originally designed to mitigate stormwater runoff and enhance buildings’ thermal insulation, their potential ecological benefits
such as increasing biodiversity have been receiving more focus in past years (Blaustein, Kadas, & Gurevitch, 2016; Lundholm & Peck, 2008; Sutton & Lambrinos, 2015). The steady increase in urbanization, alongside the popularity of green roofs, suggests a potential key role of green roofs at increasing urban biodiversity if designed correctly (Blaustein et al., 2016). Green roof studies can provide ideal testing grounds for general ecological theory (Vasl & Heim, 2016) being man-made, and they offer a high level of experimental control. The results of these studies would not only improve theoretical insights but give verified practical tools for green roof designers to implement in their green roof planning and enhance green roof biodiversity. Since green roofs are carefully designed and generally costly, simple manipulations that would stabilize and enhance a diverse plant community—for example, substrate heterogeneity could prove a highly beneficial and a cost-effective method to increase diversity on green roofs.

Green roof studies have previously targeted the enhancement of species diversity via heterogeneity. Previous studies have manipulated different substrate features (Lundholm, 2009) as well as the mixing of annuals with perennials (Vasl, Shalom, Kadas, & Blaustein, 2017), creating heterogeneous surface features such as logs and pebbles (Walker & Lundholm, 2017) and substrate depth (Heim & Lundholm, 2014).

We established green roof modules and manipulated heterogeneity of a set amount of different substrate components with relatively large subplot size. We predicted that the different substrate niches would support different plant communities which would lead to higher levels of total plant diversity in the more heterogeneous modules. In an attempt to avoid effects caused by specific kinds of heterogeneity (partially mentioned in hypothesis 1), we tested both the commonly manipulated organic components as well as nonorganic components that are commonly used in the green roof industry that have very different features (e.g., weight and water content).

We emphasize that the treatment performed in this study was only the level and type of inner distribution of the total substrate components while total substrate components were kept similar. The goal of this experiment was not to discern the effect that each of the specific treated substrate compositions has on the plant community but instead to isolate the role of substrate heterogeneity on plant diversity. However, following the results of plant communities in control and treated plots, we did analyze plant species distribution within the plot to better our understanding of the processes that took place throughout the experiment.

2 | METHODS

2.1 | Experimental design

The experiment included 24 experimental modules that were placed on three school roofs (eight modules per roof) in the city of Haifa, Israel, and monitored for three consecutive growing seasons. Haifa has a typical dry Mediterranean climate with short rainy winters and long, hot, and dry summers. Precipitation events mainly take place between late October and early April. The three schools were “Dinur,” “Ben-Gurion,” and “Matos” (Table 1). Selected schools were ones with safe access and a suitable roof sealing layer and were relatively near each other.

Assembly of all experimental modules was completed on 2 December 2013. Prior to the completion of the experimental modules, very few early rains (total of 7 mm over 6 minor rain events) occurred, so the relatively late start should have had little impact on plant development in the first growing season. Module frames (length × width × height: 2,000 × 2,000 × 200 mm) were made of wood and lined with a 0.5 mm waterproof plastic membrane sheet (Wepelen® Aqua Tec, RKW). A 2-cm deep drainage mat composed of recycled polyethylene foam waste (3RFOAM, “Palziv”) was placed on top of the waterproof plastic membrane sheet. The modules, consistent with green roof practice (FLL, 2008), were placed on a 2° slope on each of the roofs. One drainage point per module was situated 50 mm above the lower-most corner of the module. A 400 × 400 mm “cushion” made of a coated nonwoven root barrier sheet (Plantex® Gold; DuPont) containing 1 L of large tuff (4–8 mm) was placed on the inner side of the drainage unit to filter runoff water and prevent clogging of drainage. Modules were placed on a synthetic foam sheet (GalFoam – GA400, “Palziv”) to insulate the modules from the roofs and to protect the modules and the roofs from physical damage.

Substrate for all modules was composed of 10% peat, 10% compost, 10% tuff (local volcanic ash—0–8 mm), and 70% processed perlite (imported amorphous volcanic glass—0.6 mm, produced by “Agrical”). Treatments were composed of different levels of dispersion of substrate components. Treatments included the following: (a) homogeneous dispersion (i.e., “HOM”)—all components were homogeneously distributed; (b) mineral heterogeneity (i.e., “M-HET”)—only mineral components (perlite and tuff) were heterogeneous in their dispersion; (c) organic heterogeneity (i.e., “O-HET”)—only organic components (compost and peat) were heterogeneous in their dispersion; and (d)

| Table 1 | Characteristics of the three schools where experiments were placed |
|-------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| School name       | Location        | Elevation (m asl) | Precipitation (mm) | Average max daily temp. (°C) |
|                   |                 |                 | 2014–15 | 2015–16 | Jan 2015 | Aug 2015 | Jan 2016 |
| "Dinur"           | 32.79°N, 35.01°E | 186             | 577.4   | 337.5   | 19.45   | 38.19   | 18.77 |
| "Ben-Gurion"      | 32.79°N, 35.00°E | 208             | 585.6   | 345.5   | 17.34   | 34.81   | 16.94 |
| "Matos"           | 32.81°N, 34.98°E | 264             | 635.3   | 347.5   | 16.37   | 34.98   | 16.19 |

Note: Temperature and precipitation were collected for the second and third years.
mineral and organic heterogeneity (i.e., “M+O-HET”)—both mineral and organic components were heterogeneous in their dispersion. In order to retain the tuff:perlite and low:high organic matter ratios, the total sum of tuff in this treatment was slightly higher (96.19 L per module) and perlite was slightly lower (479.81 L per module) than other treatments (Table 2). All treatment compositions were achieved by mixing the individual components for a constant period of time in a clean portable electric cement mixer.

All modules were subdivided, and four subplots (each subplot: 500 × 500 mm) with plastic frames were positioned in module corners, 250 mm from the module border (Table 2). Subplot plastic frames were placed prior to the filling of the module with the substrate and removed after substrate was filled so that there was no physical barrier between the subplots and the remainder of the module. Diagonal subplots were paired, and each pair consisted 1/8 of the total module area (=0.5 m²). In treatments M-HET and O-HET, one pair of subplots (randomly chosen) was filled with the additional substrate mix. In treatment M+O-HET, the two additional substrate mixes were randomly added to the two different pairs. The remaining subplots (in treatments HOM, M-HET, and O-HET) were filled with the corresponding volume of the substrate used in the rest of the module.

Seeds of 19 species of local annuals from different families including grasses and nitrogen fixers were collected throughout 2013, and seeds of Agrostemma githago (a locally protected species) were purchased from a local wild flower nursery (“Seeds from Zion”) (Table 3). Each of the modules was seeded with a total of 4,000 seeds—200 seeds from each of the 20 species. Seeds were mixed in a bucket with 1 L of sand and evenly distributed over the entire experimental module.

Modules were then covered with a 20 mm layer of medium-sized (6–20 mm) gravel to avoid wind erosion of perlite-based substrates and seed scattering before the first rains of the first season.

### TABLE 2 Substrate compositions of the different treatments components.

| Homogeneous | Mineral heterogeneity | Organic heterogeneity | Mineral and organic heterogeneity |
|-------------|-----------------------|-----------------------|----------------------------------|
| HOM (1)     | M-HET perlite 1(2a)   | O-HET high organic   | M+O-HET matrix (4a)              |
|             | M-HET tuff (2b)       | O-HET low organic    | M+O-HET tuff (4b)                |
|             |                       |                      | M+O-HET low organic (4c)         |
| L %         | L %                   | L %                  | L %                             |
| Perlite     | 504 70                | 504 80 0             | 405 75 0                         |
| Tuff        | 72 10                 | 0 0 72 80            | 0 0 85.5 95                      |
| Compost     | 72 10                 | 63 10 9 10           | 67.5 12.5 2.25 2.5              |
| Peat        | 72 10                 | 63 10 9 10           | 67.5 12.5 2.25 2.5              |

Note: All modules contained a total of 720 L of substrate but components were dispersed differently within the different treatments. All treated subplots (one treatment for M-HET and O-HET and two for M+O-HET) were separated into two 0.5 × 0.5 m subplots that were placed 250 mm from the module edges.

2.2 | Plant development measures

2.2.1 | Point-intercept measures

In the beginning of February 2014, a nondestructive biomass measure was performed once a month throughout the growing seasons using the point-intercept method (Jonasson, 1988). One hundred metal skewers (diameter of 2.5 mm) were uniformly placed (83.3 mm apart) in each of the modules. Number and identity of green plant organs that intercepted with the skewer were documented. The sum of the yearly touches was used as a biomass proxy, and the identity was used to estimate species distributions within the module. While different growth forms have been shown to have different biomass:intercept ratios, use of this method for repeated monitoring within given experimental units containing several growth forms has been shown to be effective (Bråthen & Hagberg, 2004).

2.2.2 | Individual count

At the end of the growing period of each of the species, all dead plants were counted. These data were used to calculate total module yearly Shannon–Wiener diversity index ($H'$).

2.3 | Subplot level analysis

Point-intercept data were tracked on the subplot level so that the total sum of intercepts counted in the treated subplots (the two diagonal paired subplots—total of 18 skewers) as well as the respective “control” subplots that contained similar substrate to that in the matrix could be attained for each of the modules.

A sum of yearly species identity for each of the potential treated and “control” subplots was calculated. The seven different subplots...
one subplot value for treatment HOM, and two for each of the other three treatments—M-HET (tuff and perlite subplots), O-HET (low and high organic subplots), and the M+O-HET (tuff and low organic subplots). The point-intercept subplot communities were used to calculate Bray–Curtis distances for all three growing seasons.

### 2.4 Substrate change monitoring

Core samples (50 ml) were collected from each module at the end of each growing season after substrate was dry (18 September 2014, 20 September 2015, and 8 July 2016) to determine whether substrate composition differences were maintained over time. Two paired samples were taken from both the matrix and subplot at distance of 100 mm from either side of the initial subplot border with the module matrix. In light of the substantial weight differences between tuff and perlite, samples were initially weighed to assess changes in tuff:perlite ratios over time and then burned for 12 hr at 550°C at the Neve Ya'ar Agricultural Center to obtain percent organic matter. Since percent organic matter is a weight factor and the original substrate mixes were by volume, we could not compare percent organic matter when the two samples differed in their tuff:perlite ratio as their weight differences mask organic matter differences. For this reason, we could only use percent organic matter results for treatments HOM and O-HET. Substrate moisture (volumetric water content) was measured once a month throughout the growing seasons of the years of the study, with an ECH2O EC-5 frequency domain probe (Decagon Devices Inc.). Measurements were taken on either side (distance of 250 mm) of the initial subplot border with the module matrix. We used only the January measurements that represent the peak of the rainy season.

### 2.5 Statistical analysis

The experiment consisted of four different treatments, with six replicated modules equally distributed in three blocks, that is, two samples of each treatment on each of the three schools. Repeated measures one-way ANOVA (SPSS 23; SPSS Inc.) was performed for total plot point-intercept biomass proxy and Shannon–Wiener diversity index as well as for assessing the differences between the subplot and the module matrix (weight, moisture, and percent organic matter) throughout

---

**Table 3** Species list used in the study alongside their flowering date. Plant species used in the experiment

| Species                        | Family                  | Peak flowering | Seed collection date | Collection location |
|--------------------------------|-------------------------|----------------|----------------------|---------------------|
| 1. **Agrostemma githago** L.   | Caryophyllaceae         | April–May      | -                    | -                   |
| 2. **Anthemis pseudocotula**  Boiss. | Compositae              | March–April    | 21.8.13              | 32.71N, 34.95E      |
| 3. **Chaetoscidium trichospermum** (L.) Boiss. | Apiaceae               | March–April    | 24.4.13              | 32.80N, 35.00E      |
| 4. **Chrysanthemum coronarium** L. | Compositae              | February–April | 26.5.13              | 32.76N, 35.02E      |
| 5. **Cichorium endivia** L.    | Compositae              | April–June     | 14.11.13             | 32.76N, 35.02E      |
| 6. **Daucus broteri** Ten.     | Apiaceae                | April–June     | 2.7.13               | 32.78N, 34.97E      |
| 7. **Echi um judaemum** Lacaita | Boraginaceae            | March–April    | 12.6.13              | 32.78N, 34.97E      |
| 8. **Erodium malacoides** (L.)’L’Her. | Geraniaceae             | January–April  | 17.3–8.4.13          | 32.63N, 35.07E      |
| 9. **Heliotropium hirsutissimum** Grauer | Boraginaceae            | May–October    | 4–10.8.13            | 32.76N, 35.02E      |
| 10. **Hirschfeldia incana** (L.) Lagr.-Foss. | Brassicaceae            | January–April  | 12.6.13              | 32.76N, 35.02E      |
| 11. **Lagurus ovatus** L.      | Poaceae                 | March–April    | 21.8.13              | 32.71N, 34.94E      |
| 12. **Lomelosia prolifera** (L.) Greuter and Burdet | Dipsacaceae            | March–May      | 27.5.13              | 32.68N, 35.08E      |
| 13. **Malva parviflora** L.    | Malvaceae               | February–April | 5.4.13               | 32.63N, 35.07E      |
| 14. **Riccia lunaria** (L.) DC. | Brassicaceae            | January–April  | 15.3–15.4.13         | 32.79N, 35.01E      |
| 15. **Silene aegyptiaca** (L.) L. f. | Caryophyllaceae         | January–April  | 11–25.3.13           | 32.63N, 35.07E      |
| 16. **Sinapis alba** L.        | Brassicaceae            | January–April  | 30.5.13              | 32.77N, 35.01E      |
| 17. **Stipa capensis** Thunb.  | Poaceae                 | March–May      | 30.4.13              | 31.58N, 34.94E      |
| 18. **Tordylium carnell** (Labill.) Al-Eisawi and Juri | Apiaceae               | April–June     | 12.6.13              | 32.76N, 34.98E      |
| 19. **Trifolium purpureum** Loisel. | Fabaceae               | March–May      | 27.5.13              | 32.64N, 35.06E      |
| 20. **Trifolium stellatum** L. | Fabaceae                | February–April | 5.4.13               | 32.64N, 35.06E      |

Note: Annuals are from 10 different families including grasses (Poaceae) and nitrogen fixing legumes (Papilionaceae). All seeds were collected from wild populations except for the locally protected Agrostemma githago whose seeds were purchased.
the 3 years of the experiment. Parametric assumptions including homogeneity of variance (Levene’s test) and normal distribution (Shapiro–Wilk test) of residuals were tested.

Data were transformed (specific transformations are reported at each relevant test) when parametric assumptions were not met. Greenhouse–Geisser corrections for degrees of freedom were used when sphericity assumptions were not met.

Community dissimilarity between modules and between subplots was calculated using Bray–Curtis differences. The data were visualized in nonmetric dimensional scaling plots (NMDS), using the meta-DATA function in the vegan package of R (Oksanen et al., 2013). A nonparametric multivariate analysis of variance PERMANOVA on Bray–Curtis dissimilarities with 999 permutations was performed on whole module species abundance data and subplot community point-intercept data for each of the years using “adonis” function of “vegan” package in R, with block, treatment, and their interactions as predictors. Since PERMANOVA tests do not have post hoc procedures, when treatment was statistically significant, we performed pairwise t tests on each of the combinations to establish which were different. Critical p values were corrected following the “Benjamini–Hochberg” false discovery correction (Benjamini & Hochberg, 1995).

3 | RESULTS

3.1 | Total module results

Biomass proxy (point intercept) did not change with substrate heterogeneity, but increased from the first to the second year and decreased in the third year (Figure 1a) (repeated measures one-way ANOVA, \( p < .001 \); Table 4).

Shannon–Wiener diversity index (\( H' \)) did not change with substrate heterogeneity either, but decreased over the 3 years of the experiment (Figure 1b) (repeated measures one-way ANOVA (\( x^2 \)-transformed), \( p < .001 \); Table 4).

Plant community similarities displayed in nonmetric dimensional scaling (NMDS) in Figure 2 depict the small effect of substrate heterogeneity as opposed to the change and divergence depicted over time as well as the strong effect of school identity.

Bray–Curtis distances of whole module communities for each of the years showed a significant treatment effect only on the first year (PERMANOVA, \( p = .01 \); Table 5) while school block effects were significant on years 1 and 3 (\( p < .001 \) and \( p < .01 \), respectively; Table 5). Pairwise comparisons performed on the first-year results found that only treatments M-HET and O-HET had a significant treatment effect between them (Pseudo-F(1) = 3.03, \( p = .004 \)).

3.2 | Subplot level analysis

Biomass proxy differences between the sums of the two treated and the two control subplots showed a significant effect for treatment (Repeated measures one-way ANOVA, \( F_{2,15} = 16.84, p < .001 \)) while year (\( F_{1,23,18.38} = 1.11, p = .32 \)) and year*treatment interaction (\( F_{1,23,18.38} = 2.45, p = .07 \)) were not significant (Figure 3). Post hoc tests (Tukey’s HSD) showed that the differences between control and treated subplots in treatment M-HET were higher than those in treatments HOM and O-HET during the first 2 years.

Bray–Curtis distances of the seven subplot communities (HOM, M-HET-tuff, M-HET-perlite, O-HET-low, O-HET-high, M+O-HET-tuff, and M+O-HET-low) for each of the years (based on point-intercept data) showed a significant treatment effect only on the first year (PERMANOVA, \( p < .001 \); Table 6). School (=block) effects were significant on all 3 years (\( p = .02, p = .02, \) and \( p < .001 \) respectively; Table 6). Pairwise comparisons performed on the first year’s results (Table 7) showed that the communities present in the tuff subplots of M-HET were significantly different from all other communities excluding the communities on the tuff subplots in M+O-HET. The communities in low organic subplots in O-HET were also significantly
different from all other communities excluding the communities on low organic subplots in M+O-HET and the high organic communities in O-HET subplots.

Nonmetric multidimensional scaling (NMDS) visualization of plant communities (based on point-intercept data) in the seven different subplots (Figure 4) depicts the differences between plant communities in subplots over time.

3.3 | Substrate differences over time

Differences between core sample weights for each of the treatments (treated subplot as well as the substrate near it) (Figure 5a) found a treatment effect (repeated measures one-way ANOVA, p < .001; Table 8). Tukey’s post hoc tests showed that the tuff subplots in treatments M-HET and M+O-HET were significantly heavier than the HOM and low organic subplots from treatments O-HET and M+O-HET.

A treatment effect (p < .001; Table 8) was found for differences in January moisture measurements (repeated measures one-way ANOVA arcsin-square root-transformed) (Figure 5b). Post hoc tests showed that the moisture differences for the two tuff subplots were significantly drier while other subplots were not.

Differences between percent organic matter (arcsin-square root-transformed) of treatments HOM and O-HET showed that there was a statistically significant treatment effect (Repeated measures one-way ANOVA, p = .001) while differences were larger in O-HET subplots and that year and year*treatment interaction were not significant (Table 8, Figure 6).

### Table 4
Repeated measures ANOVA table for treatment effects on plant community biomass proxy and diversity values

| Source of variance | Point intercept | Shannon–Wiener |
|--------------------|----------------|----------------|
|                     | df  | F   | p   | df  | F   | p   |
| **Between subject** |     |     |     |     |     |     |
| Treatment          | 3.20| 0.55| 0.66| 3.20| 0.37| 0.78|
| **Within subjects**|     |     |     |     |     |     |
| Year               | 1.54| 95.1| <0.001| 2.40| 237.68| <0.001|
| Year*Treatment     | 4.61| 0.4  | 0.94| 6.40| 0.51 | 0.8  |

Note: Repeated measures one-way ANOVA on the effects on point intercept and Shannon–Wiener diversity index in the experimental modules over the 3 years of the experiment. Degrees of freedom were adjusted based on Greenhouse–Geisser adjustments. Significant results appear in bold.

### Table 5
PERMANOVA table for treatment and block effects on year module community assemblages for each year

|                | df | Pseudo-F | p   | df | Pseudo-F | p   | df | Pseudo-F | p   |
|----------------|----|----------|-----|----|----------|-----|----|----------|-----|
| **Treatment**  | 3  | 2.03     | 0.01| 2  | 0.52      | 0.09| 2  | 0.21     | 0.99|
| **Block**      | 2  | 4.86     | <0.001| 1.84| 0.09      | 0.09| 4.35| 0.005    |     |
| **Treatment*block** | 6  | 0.83     | 0.75| 0.9 | 0.57      | 0.62| 0.62| 0.85     |     |

Note: PERMANOVA results per year performed on the Bray–Curtis distances between the community assemblages in the different modules with treatment, block (school), and their interaction used as explanatory variables. Significant results appear in bold.
In our experiment, we did not find a positive effect of substrate heterogeneity on plant diversity throughout the 3 years of the experiment. The inner module documentation of plant specimen locations suggested that plants were locally affected by substrate treatments but only on the first year. Finally, substrate yearly changes were documented and suggest that differences between substrate treatments were maintained over the years.

As portrayed above, while positive HDR is a generally accepted phenomenon with strong theoretical backing, soil HDR (especially in studies comparing similar sized units) is often not positive (Stein & Kreft, 2015).

Since experimental soil studies are typically limited in size, the size of the modules used in the experiments was targeted as the potential cause to this discrepancy (Walker & Lundholm, 2017). The even smaller patches (i.e., subplot) within the experimental modules may not be large enough to sustain individuals of a different species. Presumably, if only soil experimental studies were larger in size, the patches within the experimental modules could sustain individuals from different species and the studies would show a positive HDR in accordance with general HDR findings.

A meta-analysis performed entirely on soil manipulation studies (Tamme et al., 2010) strengthened this assumption and claimed that experimental studies’ negative relationship was limited by size of experimental units. The meta-analysis contained several large-scaled presumably “experimental” studies that showed a positive HDR which allowed the researchers to reach this conclusion. However, the terminology used in this study may have been misleading, as they define “experimental” studies as binary studies with homogeneous and heterogeneous areas/modules being compared and not as commonly defined experimentally manipulated studies. As a

|         | 2014        | 2015        | 2016        |
|---------|-------------|-------------|-------------|
| Treatment | df | Pseudo-F | p | df | Pseudo-F | p | df | Pseudo-F | p |
| Treatment | 6 | 2.34 | <0.001 | 1.27 | 0.17 | 1.1 | 0.36 |
| Block | 2 | 2.07 | 0.02 | 2.15 | 0.02 | 8.63 | <0.001 |
| Treatment*block | 12 | 0.91 | 0.69 | 0.69 | 0.91 | 0.48 | 0.99 |

Note: PERMANOVA results per year performed on the Bray–Curtis distances between the community assemblages in the seven different subplots (HOM, tuff, and perlite in M-HET, low and high organic in O-HET, and tuff and low organic in M+O-HET) with treatment, block (school), and their interaction used as explanatory variables. Significant results appear in bold.

**TABLE 6**  PERMANOVA for treatment and block effects on yearly subplot community assemblages

**TABLE 7**  Pairwise PERMANOVA tests for the subplot community assemblages of 2014

|         | HOM | M-HET tuff | M-HET perlite | O-HET low organic | O-HET high organic | M+O-HET tuff |
|---------|-----|------------|---------------|-------------------|-------------------|-------------|
| M-HET tuff | 0.005 | M-HET perlite | O-HET low organic | O-HET high organic | M+O-HET tuff |
| M-HET perlite | 0.774 | 0.002 | 0.01 | 0.247 | 0.004 | 0.4 | 0.032 |
| O-HET low organic | 0.008 | 0.289 | 0.037 | 0.763 | 0.016 | 0.296 |
| O-HET high organic | 0.247 | 0.284 | 0.004 | 0.653 | 0.582 | 0.532 |
| M+O-HET tuff | 0.008 | 0.289 | 0.037 | 0.763 | 0.016 | 0.296 |
| M+O-HET low organic | 0.173 | 0.004 | 0.284 | 0.653 | 0.582 | 0.532 |

Note: *p*-Value results for pairwise PERMANOVA tests of the different subplot communities for the 2014 season (obtained from yearly point-intercept data). Test results show that treatment M-HET tuff subplots differs from all other subplot communities and O-HET low organic subplots differs from all other subplot communities with the exception of M+O-HET low organic. *p*-Values <0.05 appear in bold, and results significant after the “Benjamini–Hochberg” correction appear with a gray background.
The vast majority of the studies included were confined in their size (module < 0.25 m²) (Tamme et al., 2010) and it was not possible to successfully isolate the targeted size factor. An additional inherent problem with most soil heterogeneity studies conducted in the past was that they were often limited to only one growing season (Gazol et al., 2013; Price, Gazol, Tamme, ...
Our findings showed that community composition in treated experimental studies. These findings allow us to point toward a potential effect on the community level that was not previously explicitly examined in experiments.

This study was kindly supported by the Kadas family and Haifa University.
CONFLICT OF INTEREST
All authors declare they have no conflict of interest.

AUTHORS CONTRIBUTION
AV, GJK, and LB conceived the ideas and designed methodology; AV and BYS collected the data; AV and BYS analyzed the data; AV led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT
Sampling data and r script: https://doi.org/10.5061/dryad.86qh1tm.

ORCID
Amiel Vasl https://orcid.org/0000-0003-0874-0858

REFERENCES
Baer, S. G., Blair, J. M., & Collins, S. L. (2015). Environmental heterogeneity has a weak effect on diversity during community assembly in tallgrass prairie. Ecological Monographs, 86, 94–106. https://doi.org/10.1890/15-0888.1
Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. Journal of the Royal Statistical Society, Series B (Methodological), 57(1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x
Blaustein, L., Kadas, G. J., & Gurevitch, J. (2016). Integrating ecology into green roof research. Israel Journal of Ecology and Evolution, 62, 1–6. https://doi.org/10.1080/15659801.2016.1208943
Braaker, S., Ghazoul, J., Obrist, M. K., & Moretti, M. (2014). Habitat connectivity shapes urban arthropod communities: The key role of green roofs. Ecology, 95, 1010–1021. https://doi.org/10.1890/13-0705.1
Bråthen, K. A., & Hagberg, O. (2004). More efficient estimation of plant biomass. Journal of Vegetation Science, 15, 653–660. https://doi.org/10.1111/j.1654-1103.2004.tb02307.x
Chesson, P. (2000). Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics, 31, 343–366. https://doi.org/10.1146/annurev.ecolsys.31.1.343
FLL (2008). Guidelines for the planning, construction and maintenance of green roofing – Green roofing guideline. The Landscape Development and Landscaping Research Society e.V. (FLL), Germany.
Gazol, A., Tamme, R., Price, J. N., Hiiesalu, I., Laanisto, L., & Pärtel, M. (2013). A negative heterogeneity – Diversity relationship found in experimental grassland communities. Oecologia, 173, 545–555. https://doi.org/10.1007/s00442-013-2623-x
Heim, A., & Lundholm, J. (2014). The effects of substrate depth heterogeneity on plant species coexistence in an extensive green roof. Ecological Engineering, 68, 184–188.
Hubbell, S. P. (2001). The unified neutral theory of biodiversity and biogeography. Princeton, NJ: Princeton University Press.
Hutchings, M. J., John, E. A., & Wijesinghe, D. K. (2003). Toward understanding the consequences of soil heterogeneity for plant populations and communities. Ecology, 84, 2322–2334. https://doi.org/10.1890/02-0290
Jonasson, S. (1988). Evaluation of the point intercept method for the estimation of plant biomass. Oikos, 52, 101–106. https://doi.org/10.2307/3565988
Kadmon, R., & Allouche, O. (2007). Integrating the effects of area, isolation, and habitat heterogeneity on species diversity: A unification of island biogeography and niche theory. American Naturalist, 170, 443–454. https://doi.org/10.1086/519853
Leibold, M. A., & McPeek, M. A. (2006). Coexistence of the niche and neutral perspectives in community ecology. Ecology, 87, 1399–1410. https://doi.org/10.1890/0012-9658(2006)87[1399:COTNA]
N2.0.CO.2
Lundholm, J. T. (2009). Plant species diversity and environmental heterogeneity: Spatial scale and competing hypotheses. Journal of Vegetation Science, 20, 377–391. https://doi.org/10.1111/j.1654-1103.2009.05577.x
Lundholm, J. T., & Peck, S. W. (2008). Introduction: Frontiers of green roof ecology. Urban Ecosystems, 11, 335–337. https://doi.org/10.1007/s11252-008-0070-y
MacArthur, R., & Levins, R. (1964). Competition, habitat selection, and character displacement in a patchy environment. Proceedings of the National Academy of Science of the USA, 51, 1207–1210.
Matthews, T. J., & Whittaker, R. J. (2014). Neutral theory and the species abundance distribution: Recent developments and prospects for unifying niche and neutral perspectives. Ecology and Evolution, 4, 2263–2277. https://doi.org/10.1002/ece3.1092
Oberndorfer, E., Lundholm, J., Bass, B., Coffman, R. R., Doshl, H., Dunnett, N., ... Rowe, B. (2007). Green roofs as urban ecosystems: Ecological structures, functions, and services. BioScience, 57, 823–833. https://doi.org/10.1641/BS10050
Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O’hara, R. B., ... Oksanen, M. J. (2013). Package ‘vegan’. Community ecology package, version, 2(9), 1–295.
Palmer, M. W. (1992). The coexistence of species in fractal landscapes. The American Naturalist, 139(2), 375–397. https://doi.org/10.1080/285332
Price, J. N., Gazol, A., Tamme, R., Hiiesalu, I., & Pärtel, M. (2014). The functional assembly of experimental grasslands in relation to fertility and resource heterogeneity. Functional Ecology, 28, 509–519. https://doi.org/10.1111/1365-2435.12186
Rajaniemi, T. K. (2011). Competition for patchy soil resources reduces community evenness. Oecologia, 165, 169–174. https://doi.org/10.1007/s00442-010-1710-5
Shmida, A., & Ellner, S. (1984). Coexistence of plant species with similar niches. Vegetatio, 58, 29–55.
Smith, T. W., & Lundholm, J. T. (2012). Environmental geometry and heterogeneity–diversity relationships in spatially explicit simulated communities. Journal of Vegetation Science, 23, 732–744. https://doi.org/10.1111/j.1654-1103.2011.01380.x
Stein, A., Gerstenk, N., & Kreft, H. (2014). Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales. Ecology Letters, 17, 866–880. https://doi.org/10.1111/ele.12277
Stein, A., & Kreft, H. (2015). Terminology and quantification of environmental heterogeneity in species-richness research. Biological Reviews, 90, 815–836. https://doi.org/10.1111/brv.12135
Sutton, R. K., & Lambbrinos, J. (2015). Green roof ecosystems: Summary and synthesis. In R. K. Sutton (Ed.), Green roof ecosystems (pp. 423–440). Basel, Switzerland: Springer.
Tamme, R., Gazol, A., Price, J. N., Hiiesalu, I., & Pärtel, M. (2016). Co-occurring grassland species vary in their responses to fine-scale soil heterogeneity. Journal of Vegetation Science, 27, 1012–1022. https://doi.org/10.1111/jvs.12431
Tamme, R., Hiiesalu, I., Laanisto, L., Szava-Kovats, R., & Pärtel, M. (2010). Environmental heterogeneity, species diversity and co-existence at different spatial scales. Journal of Vegetation Science, 21, 796–801. https://doi.org/10.1111/j.1654-1103.2010.01185.x
Vasi, A., & Heim, A. (2016). Preserving plant diversity on extensive green roofs—theory to practice. Israel Journal of Ecology and Evolution, 62, 103–111. https://doi.org/10.1080/15659801.2015.1035507
Vasl, A., Shalom, H., Kadas, G. J., & Blaustein, L. (2017). Sedum—Annual plant interactions on green roofs: Facilitation, competition and exclusion. Ecological Engineering, 108, 318–329. https://doi.org/10.1016/j.ecoleng.2017.07.034

Walker, E. A., & Lundholm, J. T. (2017). Designed habitat heterogeneity on green roofs increases seedling survival but not plant species diversity. Journal of Applied Ecology, 55(2), 694–704. https://doi.org/10.1111/1365-2664.12970

Whittaker, R. H. (1965). Dominance and diversity in land plant communities: Numerical relations of species express the importance of competition in community function and evolution. Science, 147, 250–260. https://doi.org/10.1126/science.147.3655.250

Williams, B. M., & Houseman, G. R. (2014). Experimental evidence that soil heterogeneity enhances plant diversity during community assembly. Journal of Plant Ecology, 7, 461–469. https://doi.org/10.1093/jpe/rtt056