1 **Soil invertebrates in Australian rain gardens and their potential roles in storage and**

2 **processing of nitrogen**

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4 Andrew S. Mehring[1*], Belinda E. Hatt[2], Diana Kraikittikun[1], Barbara D. Orelo[1], Megan A.

5 Rippy[3], Stanley B. Grant[3], Jennifer P. Gonzalez[1], Sunny C. Jiang[3], Richard F. Ambrose[4], Lisa A.

6 Levin[1]

7

8 [1]Integrative Oceanography Division and Center for Marine Biodiversity and Conservation,

9 Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA, 92093,

10 USA

11 [2]Department of Civil Engineering and Monash Water for Liveability, 23 College Walk, Monash

12 University, Victoria, Australia

13 [3]Department of Civil and Environmental Engineering, Henry Samueli School of Engineering,

14 University of California, Irvine, Irvine, CA, 92697, USA

15 [4]Department of Environmental Health Sciences, University of California, Los Angeles, Los

16 Angeles, CA, 90095, USA

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18 *Correspondence: Email: amehring@ucsd.edu, Phone: 858-534-3579

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Highlights

- Rain garden soil invertebrate communities differed spatially but not temporally

- Oligochaetes and springtails accounted for 80% of total soil invertebrate abundance

- Earthworm abundance was positively correlated to soil organic matter content

- Estimated soil invertebrate N storage and gas emissions were low but estimated ingestion rates were high

- Intersite differences in invertebrate functional effects may be temporally stable

Keywords

Biofilter, bioretention system, low impact development, LID, Lumbricidae, WSUD

Abstract

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

45   site), suggesting that the contribution of these organisms to N cycling in rain gardens may be

46   substantial. Thus, invertebrate communities represent an overlooked feature of rain garden

47   design that can play an important role in the structure and function of these systems.

48   **1. Introduction**

49   Rain gardens (also known as biofilters and bioretention systems) are small, terrestrial

50   natural treatment systems designed to filter pollutants from stormwater using porous filter media

51   planted with one or more species of vegetation (Ambrose and Winfrey 2015; Askarizadeh and

52   others 2015). Soon after rain garden construction, soil invertebrate communities develop (Ayers

53   2009) that may impact important rain garden functions such as infiltration and nutrient retention

54   or removal (Levin and Mehring 2015; Mehring and Levin 2015). Though it has yet to be tested

55   in the context of rain gardens, substantial amounts of carbon (C), nitrogen (N) and phosphorous

56   (P) may be stored in soil invertebrate biomass (Teuben and Verhoef 1992), which temporarily

57   immobilizes nutrients and prevents them from being leached. Soil invertebrates may also impact

58   nutrient retention indirectly through increasing nutrient uptake by plants. The effects of

59   earthworm activity on plant uptake of N are particularly dramatic, with some species reported to

60   enhance uptake > 200% in vertical-flow wetlands (Xu and others 2013). Springtails, millipedes

61   (Diplopoda), and isopods also have the potential to enhance plant uptake of N due to high levels

62   of plant-available N in their fecal material (Anderson and others 1983; Teuben and Roelofsma

63   1990).

64   Soil invertebrates may play an important role in nutrient removal in rain gardens as well

65   as nutrient immobilization/retention. Earthworms, for instance, have the potential to increase

66   nitrate removal via denitrification because anoxic conditions within their guts favor production of

67   dinitrogen (N_2) and nitrous oxide (N_2O) by ingested soil-derived microbes (Horn and others

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68 2006), even when surrounding soil conditions are aerobic. Indeed earthworm casts themselves

69 may be denitrification hotspots, prolonging the effects of "worm facilitated" N removal long after

70 excretion (Parkin and Berry 1994).

71 Despite their notable effects on soil biogeochemistry, few studies to date have quantified

72 soil invertebrates in rain gardens. Those studies that have, emphasize above-ground invertebrates

73 (Kazemi and others 2009a; Kazemi and others 2009b), leaving within-soil diversity (and

74 biogeochemical roles) in rain gardens largely unknown. Here we present results from one of the

75 first studies of below-ground invertebrate communities in rain gardens. Our study took place in

76 July 2013 and April 2014 in Melbourne, Australia, and was designed to (1) assess spatial and

77 temporal patterns in invertebrate community structure within rain gardens (2) identify drivers of

78 invertebrate abundance within raingardens (e.g., vegetation type, vegetation cover, and soil

79 organic matter content), and (3) determine if soil invertebrates are likely to contribute

80 substantially to nutrient retention/removal within rain gardens (based on the nutrient content in

81 their biomass, soil processing capacity, and estimates of their contribution to denitrification).

82 **2. Methods**

83 *2.1 Study sites* **–** Three rain gardens near Melbourne, Victoria (Australia) were sampled in the

84 winter of 2013 (July) and autumn of 2014 (March-April): (1) "Hereford Road" (HR) is a 100-m^2

85 rain garden that treats runoff from a 0.93-ha peri-urban catchment in the town of Mt. Evelyn; (2)

86 "Wicks Reserve" (WR) is a 1900 m^2 rain garden that treats runoff from a 11.43-ha peri-urban

87 catchment in the town of The Basin; and (3) "Lynbrook Boulevard" (LB) is a bioretention swale

88 that treats runoff from a 2.0-ha suburban development known as Lynbrook Estate. More

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89   information on these rain gardens can be found in Supplementary Material (Supplementary

90   Material, Table S2).

91   *2.2 Field Sampling Methods* – Samples were collected at points evenly-spaced along a transect,

92   from the inlet (where stormwater flows into the rain garden) to the outlet (where excess water

93   leaves the rain garden). At least four points were sampled in each site per sampling season, with

94   9, 10, and 13 points sampled in total at LB, WR, and HR, respectively. At each sampling

95   location, a 0.25m x 0.25m quadrat was used to determine the percent cover of three commonly-

96   planted vegetation types: grasses, sedges, and rushes. Filter media samples (top 5 cm of soil

97   media) were collected from within the quadrat using a plexiglass corer 5 cm in diameter. Filter

98   media cores were fixed in 10% phosphate-buffered formalin within 8 hours of collection, and

99   shipped to the University of California, San Diego (UCSD) for analysis of invertebrate

100 community structure and filter media organic matter content. Additional sub-samples of filter

101 media were collected and composited into glass sample jars for future nitrogen content analysis.

102 Composite samples came from two depths (< 2 cm and ~ 10 cm). All composites were frozen

103 within 8 hours of collection and stored at -20ºC prior to analysis.

104 *2.3 Laboratory Methods* - Formalin-preserved filter media were rinsed over nested sieves to

105 separate invertebrates, organic matter, roots, and inorganic matter into three size fractions: 1) > 2

106 mm, 2) 0.3 - 2 mm, and 3) 0.045–0.3 mm. Soil size fractions ≥ 0.3 mm were sorted under a

107 Wild M5A stereomicroscope at 12x magnification in order to remove all invertebrates, which

108 were then classified according to order, suborder, or family, and enumerated. The most abundant

109 invertebrates (Oligochaetes, including Megadrilacea [earthworms] and Enchytraeidae

110 [potworms]; and Collembola [springtails]) were dried and individual weights were measured in

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111   order to estimate biomass per site, body tissue N content, and ingestion rates. Tissue N content

112   and ingestion rates by oligochaetes were estimated using conversion factors (see Appendix S2).

113   Following removal of invertebrates and roots, each filter media size fraction was dried to a

114   constant weight at 60°C, combusted at 500°C, and re-weighed in order to estimate soil organic

115   matter content as ash-free dry mass (AFDM). Frozen composite filter media samples (2 depths)

116   were sent to a NATA accredited laboratory (http://www.nata.asn.au/) for analysis of total N

117   using standard methods and quality assurance procedures (APHA 2012).

118   *2.4 Statistical Analysis* - Overall invertebrate community composition was explored using

119   multivariate analyses (MDS, ANOSIM, SIMPER) run using PRIMER 6 (Primer-E 2006,

120   Plymouth Marine Laboratory, Clarke 1993, Clarke & Warwick 1994) on fourth-root

121   transformed, unstandardized data (data provided in Supplementary Material, Table S1). Using

122   the same software, patterns of species richness among rain gardens were compared by using a

123   sample-based rarefaction procedure ('DIVERSE', 'Rarefaction'), where number of individual

124   invertebrates collected are set as samples and estimated species richness is calculated for a given

125   sample size. Taxonomic richness was estimated repeatedly for increasing sample sizes in each

126   site, at increments of 5 individuals, until the total number of invertebrates collected within a

127   given site was reached. If an asymptote in expected taxonomic richness is not reached for a given

128   site, it suggests that actual taxonomic richness is higher than that estimated from the sampling

129   effort.

130   Two-factor Analysis of Variance (ANOVA) was used to examine differences in

131   abundance of earthworms, potworms, springtails, and Acari (mites) among sites and between

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132   sampling dates (seasons). Linear regression was used to test for correlations between abundance

133   of these taxonomic groups and plant cover, filter media organic matter, and root biomass.

134   The contribution of invertebrate communities to nine important rain garden functions

135   (plant growth, water infiltration, plant pathogen removal, denitrification, nutrient uptake in

136   plants, nutrient storage in soil, heavy metal uptake by plants, coarse organic matter shredding,

137   and decomposition) was estimated as follows. Briefly, the average abundance (from this study)

138   of each taxonomic group capable of performing a function (Supplementary Material, Table S1)

139   was multiplied by a biomass correction factor based on body size. These values were summed

140   and then multiplied by the number of contributing taxa present in our samples, positively

141   weighting richness. The resultant scores were binned logarithmically so that function scores

142   spanning multiple orders of magnitude could be compared (see supplementary material,

143   Appendix S1 for calculations).

144   A Monte Carlo framework (Mehring and others [2015]) was used to further evaluate a

145   subset of the above-noted invertebrate functions in rain gardens concerning nitrogen.

146   Specifically, we quantify (a) the percentage of total soil N in the tissues of earthworms,

147   potworms, and springtails, (b) the amount of soil media ingested by dominant soil invertebrates

148   (earthworms and potworms) in a single year, and (c) areal rates (m^{-2} h^{-1}) of N_2O and N_2 emission

149   from earthworms in each rain garden (see Appendix S2 for calculations). This approach required

150   1) our measurements of taxon-specific abundance and biomass from each rain garden; 2)

151   parameter ranges from literature for the tissue N content of earthworms (Schmidt and others

152   1999), potworms (Pandian and Marian 1985), and springtails (Chahartaghi and others 2005); 3)

153   soil ingestion rates by earthworms (Curry and Schmidt 2007) and potworms (McBrayer and

154   Reichle 1971); and 4) N_2O and N_2 emission by earthworms (Depkat-Jakob and others 2012;

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155 Horn and others 2006; Karsten and Drake 1997; Matthies and others 1999). Note that our range

156 of earthworm soil ingestion rates is limited to values from sandy soils and mull. Because Monte

157 Carlo techniques were employed, our final estimates of invertebrate tissue N content, soil

158 ingestion, and NO_2 and N_2 emission for each rain garden reflect both variability in literature

159 parameters and our empirical measurements of abundance and biomass. These analyses were

160 conducted using R software (R Development Core Team 2008).

161 **3. Results**

162 *3.1 Spatial and Temporal Patterns in Invertebrate Community Structure* - Analysis of 32 soil

163 cores revealed diverse communities of soil invertebrates (Table S1). The highest invertebrate

164 abundance was at WR (Fig. 1A), the highest biomass was at HR, reflecting the prevalence of

165 large earthworms (Fig. 1B), and the highest taxonomic richness was found at LB, the oldest site

166 (Figure 1C). Multivariate analyses of invertebrate assemblages show significant community

167 differences among rain gardens (Fig.1D, Table 1, ANOSIM, all $p < 0.05$), but not sampling

168 dates, the exception being HR (Table 1, ANOSIM, $p = 0.014$). Community structure at LB

169 diverged most strongly from the structure observed at other rain garden sites (SIMPER, > 68%

170 dissimilar to other sites in Winter 2013 and Autumn 2014) (Fig. 1D, Table 1).

171 The most abundant invertebrates sampled across all rain gardens were potworms, earthworms,

172 springtails, and mites (Table S1, Fig. 1A), which accounted for a median of 80% of total

173 invertebrate abundance. However, significant inter-site differences in taxonomic group

174 abundance were observed. For instance, although oligochaetes in the family Naididae were

175 among the most abundant organisms at WR, they were not detected at other sites (Table S1).

176 Potworm abundance was also significantly higher at WR, while earthworm abundance was

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177 higher at HR (Tukey HSD, all p < 0.05). Neither earthworm nor potworm abundances changed

178 significantly between sampling dates, and relationships among rain gardens were stable over

179 time (no site × date interaction). Springtail abundance, however, was significantly higher in

180 Autumn 2014 than in Winter 2013 ($F_{1,26} = 5.47$, $p < 0.05$), with no significant differences among

181 sites. Mite abundance was the most stable, and did not vary significantly among sites or over

182 time.

183 *3.2 Correlates of Invertebrate Abundance Within Rain Gardens* - Across all sites, earthworm

184 abundance was positively correlated with fine organic matter (e.g., the percentage of total soil

185 dry weight composed of organic particles 0.045-2 mm in size) ($t_{1,30} = 2.91$, $R^2 = 0.22$, $p < 0.01$).

186 Mite, springtail, and potworm abundance across sites could not be explained by variations in soil

187 organic matter content, plant cover, or root biomass. However, a site-specific positive correlation

188 between potworm abundance and root biomass was observed at LB ($t_{1,7} = 2.95$, $R^2 = 0.55$, $p <$

189 0.05), and WR ($t_{1,8} = 2.23$, $R^2 = 0.39$, $p = 0.056$).

190 *3.3 Invertebrates and Rain Garden Function* – Potential invertebrate community contributions

191 to rain garden function were highest in HR (Fig. 2), followed by WR and LB. Soil invertebrates

192 in HR had high function scores (in decreasing order) for decomposition, coarse organic matter

193 (e.g. leaf litter, wood) fragmentation, nutrient uptake into plants, plant growth, and water

194 infiltration. Scores for similar functions were elevated inWR and LB, but to a lesser degree. An

195 exception is coarse organic matter fragmentation, which had relatively low scores at WR and LB,

196 due to limited abundance (or absence) of several litter transforming taxa (sensu Lavelle 1997).

197 Total soil N concentrations in HR, LB, and WR were 5100, 2900, and 460 mg kg^{-1} at < 2 cm

198 depth, and significantly lower (1400, 1000, and 140 mg kg^{-1}) at 10 cm depth (paired $t_2 = 4.42$, $p <$

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199 0.05). Our estimates suggest that <5% of depth-averaged total soil N was contained within the

200 body tissues of earthworms, potworms, and springtails > 0.3 mm in size. The contribution of

201 body tissues to soil N was particularly low at LB (< 0.05%).

202   Median estimates of areal N_2O and N_2 emissions from earthworms were low (max of 0.12

203 µg N_2O-N m^{-2} h^{-1} and 0.23 µg N_2-N m^{-2} h^{-1}), and decreased as follows: HR > WR > LB (Fig. 3A).

204 The median estimate of soil ingestion (expressed as the percent total soil mass ingested annually

205 by earthworms and potworms in the upper 5 cm of soil) was also higher at HR than at WR (43%,

206 range: <1 - 417% y^{-1} vs 4%, range: < 1 – 28% y^{-1}), and negligible at LB (<1%, range: 0-3% y^{-1})

207 (Fig. 3B). Note that estimates as high as 137% y^{-1} fell within the interquartile range at HR.

208 Because preservation in 10% formalin can cause reductions in dry mass of invertebrates (Leuven

209 and others 1985), our estimates of biomass, and therefore N storage, N_2 and N_2O emission, and

210 soil ingestion rates are likely to be conservative..

211 **4. Discussion**

212   The findings presented here suggest that invertebrate community structure may differ

213 substantially among rain gardens within a region, and that these differences are fairly stable over

214 short (~9 month) time scales. Apart from a positive correlation between earthworm abundance

215 and soil organic matter content (22% of the variation explained), we were unable to isolate

216 factors correlated with the abundance of the most common soil taxa across sites. However,

217 typical annual rainfall was higher at WR and HR than at LB (1100, 1000 and 750 mm,

218 respectively) and the presence of aquatic organisms (Chironomidae, Ostracoda, Naididae) at

219 WR, and desiccation-intolerant terrestrial amphipods (Maraldo and others 2009; Spicer and

220 others 1987) at HR, suggests that soil moisture may contribute to inter-site variability in

221 invertebrate abundance. In addition, particle size distributions, which were originally dominated

222 by gravel at LB, may have influenced early development of community structure.

223   The contribution of invertebrates to specific rain garden functions varied significantly by

224 site (Fig. 2)—a pattern that can be explained mostly by site-to-site variability in the abundance of

225 earthworms, which are well documented engineers of ecosystem services and function in soils

226 (Blouin and others 2013). Although < 5% of total filter media N was estimated to be stored in

227 invertebrate tissues per rain garden, invertebrates may stimulate N storage in alternative

228 reservoirs. For instance, soil invertebrate populations turn over quite rapidly (e.g. 1-3× per year

229 for earthworms [Parmelee and Crossley 1988]), and upon decomposition release bioavailable N

230 that is readily assimilated by plants (Whalen and others 1999). Indeed, flux of N through

231 earthworm biomass has been estimated to contribute up to 38% of plant N assimilation in some

232 agricultural systems (Parmelee and Crossley 1988). Future studies should focus on quantifying

233 invertebrate secondary production and population turnover in rain gardens, as these unknowns

234 are required to accurately assess the contribution of invertebrate-facilitated storage to total N

235 retention in these systems.

236   Denitrification within the anoxic guts of earthworms accounts for < 2.2% of average N_2O

237 emissions from rain gardens designed with and without submerged zones (Grover and others

238 2013). However, because our estimates of invertebrate-facilitated denitrification do not account

239 for enhanced denitrification in the walls of earthworm burrows (Parkin and Berry 1999) or

240 excreted soils (Parkin and Berry 1994), they are conservative, and may substantially

241 underestimate invertebrate contribution to total N removal. Indeed, our estimates of soil

242 ingestion by earthworms and potworms in rain gardens suggest that the total soil processing

243 capacity is large (up to 4× the available near-surface soil mass in a given year [Fig. 3B]).

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244 Because the potential for enhanced denitrification by earthworms scales with the mass of

245 ingested soil, their impacts on N removal in rain gardens may dramatically exceed that implied

246 by our within-worm estimates of N_2O and N_2 emission.

247   In summary, the findings presented here suggest that rain garden soil invertebrate

248 communities are diverse, stable over short timeframes (~ 9 months), vary among sites, and likely

249 impact many important rain garden functions. Rigorous assessment of soil invertebrate

250 population dynamics in rain gardens is necessary to clarify these functional roles and their

251 importance for contaminant treatment efficacy. Perhaps the most important take-home points

252 from our study are that: (1) invertebrate assemblages are site-specific, suggesting that

253 engineering design, age, and other site-associated characteristics strongly influence their

254 abundance and diversity; and (2) invertebrate functional roles in rain gardens are also site-

255 specific, with generally better outcomes expected for systems with higher abundance of

256 ecosystem engineers (such as earthworms). These findings suggest that, with improved

257 understanding of the factors that favor colonization of some taxa over others, rain garden design

258 and maintenance could be "tuned" to favor invertebrates that confer important functional traits

259 which, in turn, may improve the utility of these systems for protecting aquatic ecosystems and

260 improving human and ecosystem water security.

261 **Acknowledgements**

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271 **Table 1.** Comparisons of soil invertebrate (≥ 0.3 mm) assemblages of Hereford Road (HR), Wicks Reserve (WR), and Lynbrook

272 Boulevard (LB) rain gardens sampled in July 2013 (Winter) and March-April 2014 (Autumn). Probabilities resulting from pair-wise

273 analysis of similarity (ANOSIM) tests for invertebrate community similarities between sites are given above the diagonal (*n.s.* = *p* >

274 0.05). Values on the diagonal are percent similarity within site (SIMPER); values below the diagonal are percent dissimilarity between

275 sites (SIMPER). Significance was set at α = 0.05.

SITE / SEASON	WR Winter	WR Autumn	HR Winter	HR Autumn	LB Winter	LB Autumn
WR Winter	65.92%	n.s.	0.001	0.029	0.008	0.029
WR Autumn	43.96%	51.39%	0.005	0.010	0.002	0.005
HR Winter	59.66%	52.44%	59.52%	0.014	0.001	0.001
HR Autumn	64.32%	60.51%	53.17%	45.50%	0.016	0.029
LB Winter	68.09%	74.70%	75.24%	70.23%	38.64%	n.s.
LB Autumn	72.90%	74.59%	75.37%	70.59%	57.79%	46.04%

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278   **Figure legends.**

279   **Figure 1. (A)** Average Megadrilacea (earthworm), Enchytraeidae (potworm), Collembola

280   (springtail), and Acari (mite) abundance and **(B)** earthworm, potworm, and springtail biomass

281   per site. **(C)** Individual-based species accumulation curves and **(D)** multidimensional scaling

282   (MDS) plot of soil invertebrate ($\geq$ 0.3 mm) assemblages in Hereford Road (HR), Wicks Reserve

283   (WR), and Lynbrook Boulevard (LB) rain gardens sampled in July 2013 (Winter) and March-

284   April 2014 (Autumn).

285   **Figure 2.** Potential soil invertebrate community contributions to nine selected rain garden

286   functions in Hereford Road (HR), Wicks Reserve (WR), and Lynbrook Boulevard (LB). Each

287   function is represented by 1/9 of the pie chart and is computed for each site using the average

288   abundance of each taxonomic group known to contribute to the given function (supplementary

289   material, Table S1) multiplied by a body size correction factor. Resulting products per function

290   and site were summed and either multiplied by the number of taxa known to positively affect the

291   function of interest ("richness-weighted", left column) or not weighted by richness (right

292   column), and sorted into logarithmic bins (see supplementary material, Appendix S1 for

293   calculations). The resulting values combine species richness, biomass, and abundance into a

294   score for potential invertebrate community effect on rain garden function. Higher values within a

295   column indicate a greater potential enhancement of the given function of interest among sites.

296   Note that scales differ between columns.

297   **Figure 3. (A)** Box and whisker plots of estimated daily N_2O and N_2 emissions by earthworms.

298   Error bars represent 95% quantiles. **(B)** Box and whisker plots of estimated percentage (by dry

299   weight) of top 5 cm of rain garden soil media ingested by earthworms and potworms annually.
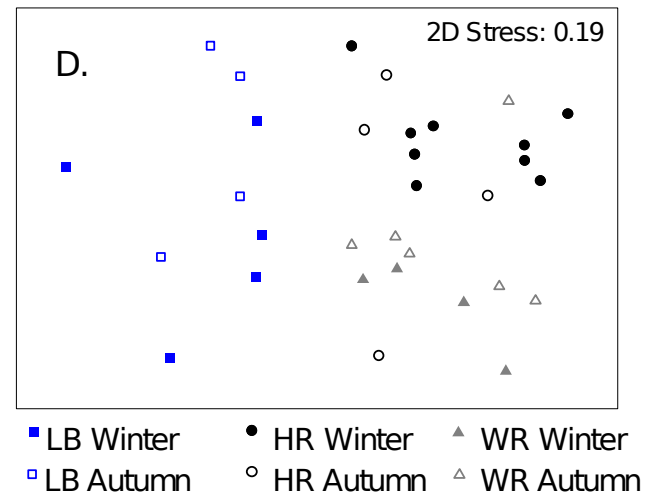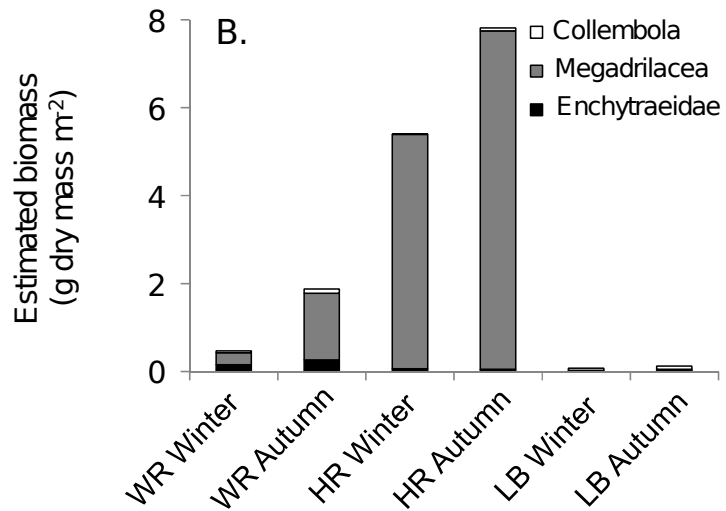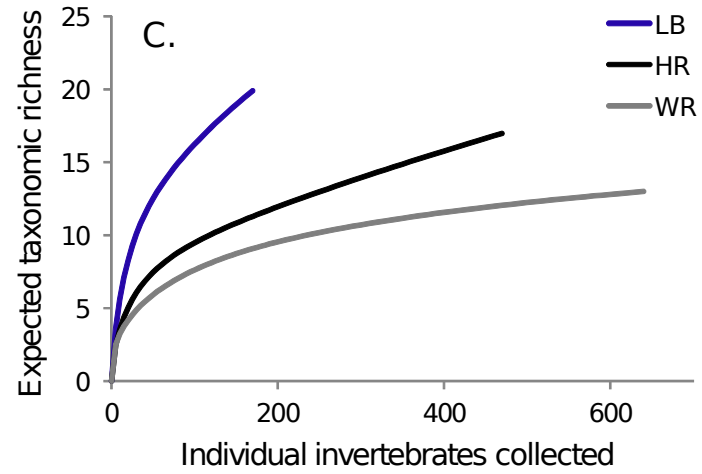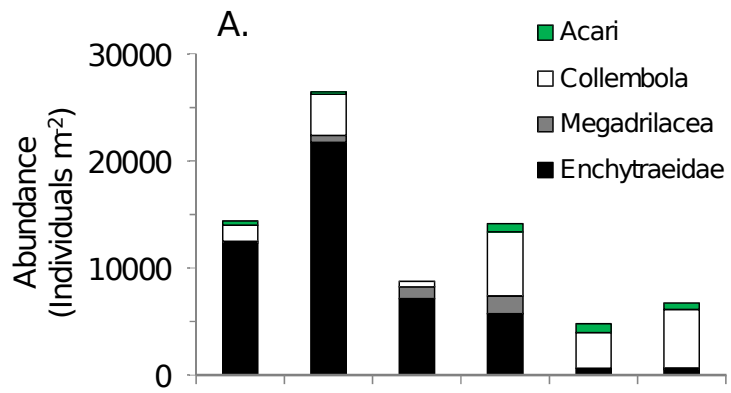
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300   Error bars represent 95% quantiles. Calculations are provided in supplementary material,
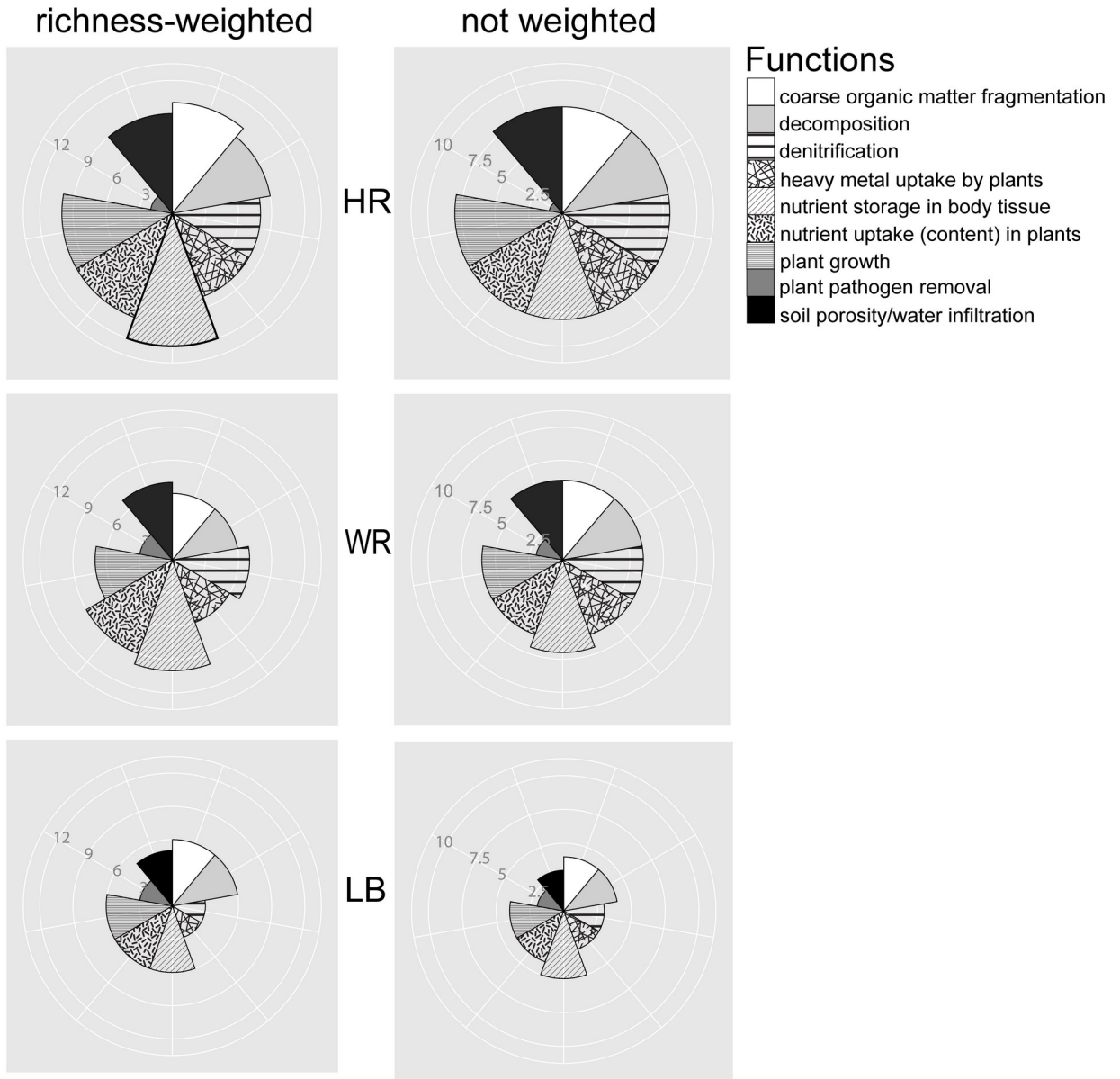
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303   **Figure 1.**
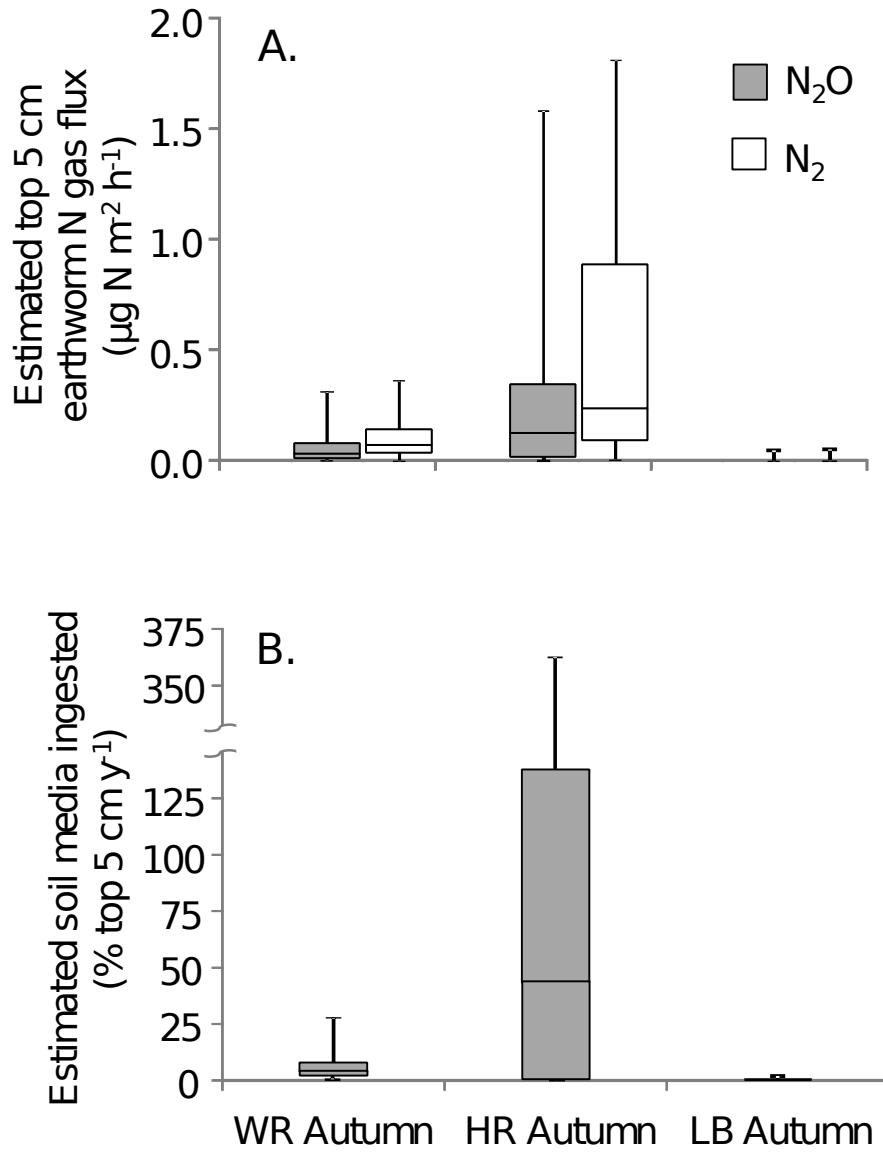
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Figure 2.

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318 **Figure 3.**

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