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Exotic herbaceous species interact with severe drought to alter soil N cycling in a semi-arid shrubland

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1 Title: Exotic herbaceous species interact with severe drought to alter soil N cycling in a semi-
2 arid shrubland

3

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18

19 **Keywords:** Climate change, Exotic species invasion, Coastal sage scrub; Soil nitrogen

20 **Abstract**

21 Mediterranean-type ecosystems are increasingly threatened by climate change and exotic annual
22 species, jeopardizing the native communities and their global biodiversity. In these systems, soil
23 nitrogen (N) limits net primary production, and its availability can be influenced by both of these
24 stressors. To understand the interactive effects of droughts and exotic herbaceous species on soil
25 N, we monitored the temporal variability of soil inorganic N, net N mineralization, net
26 nitrification, and N leaching under native- and exotic-dominated stands exposed to rainfall
27 manipulation plots in a Mediterranean-type shrub-dominated community. Increasing drought
28 severity resulted in the accumulation of soil NH_4^+ and NO_3^- , with a more pronounced increase in
29 exotic-dominated plots. **Increased net N mineralization and net nitrification** and reduced leaching
30 losses were observed as mechanisms of inorganic N accumulation. In comparison to soils under
31 native plants, soils under exotic plants had enhanced leaching losses upon soil rewetting. We
32 propose that distinct traits of exotic annual herbaceous species associated with higher N inputs,
33 faster turnover, and reduced temporal uptake determine the changes in N cycling in response to
34 droughts. Severe droughts and exotic plants may produce a larger, more vulnerable pool of N
35 that is prone to losses while providing a competitive advantage to promote exotic growth in these
36 N-limited ecosystems.

37

38 **Introduction**

39

40 Nitrogen (N) cycling is being altered globally more than any other essential nutrient
41 (Fields, 2004). Agriculture and fossil fuel combustion are increasing reactive N in the

42 atmosphere (Galloway et al., 2008), droughts are increasing the ecosystems' N losses (Homyak
43 et al., 2017), and invasive plants are altering ecosystems' N pools and fluxes (Ehrenfeld, 2003;
44 Liao et al., 2008). These N alterations represent a major challenge particularly in ecosystems
45 where N limits net primary production (Delgado-Baquerizo et al., 2014), and its availability is
46 being threatened by multiple co-occurring global change drivers (Chapin et al., 2000; Sala et al.,
47 2000). For example, semi-arid shrublands are currently experiencing invasions by exotic
48 herbaceous species (Minnich and Dezzani, 1998; Yu et al., 2016), more intense and prolonged
49 droughts (Spinoni et al., 2014; Swain et al., 2018), and elevated N deposition (Valliere et al.,
50 2017), constituting multiple stressors that may have large impacts on N cycling.

51

52 In semi-arid ecosystems, N's limited accessibility is controlled by the temporal dynamics
53 of water availability (Reichmann et al., 2013; Shen et al., 2008). During wet periods, N becomes
54 accessible for microbial and plant assimilation and transformations, which contrasts to natural
55 periods of drought when decreases in diffusion and biological uptake result in the accumulation
56 of immobile N replenished from dead biomass and dry deposition (Cregger et al., 2014). The
57 synchronized immobilization by microbes and plants during wet periods coupled with limited
58 diffusivity during dry periods promotes a closed N cycle (recycling) in these systems (Homyak et
59 al., 2016; Joergensen and Wichern, 2018). However, an asynchrony/decoupling between soil N
60 availability and plant and microbial processes creates N leaks, particularly when dry soils rewet
61 outside of the plant growing season (Homyak et al., 2016). Drought-tolerant microbial
62 transformations and a lack of plant demand during ephemeral periods with mobile N availability
63 favors N losses via leaching and gaseous pathways (Dijkstra et al., 2012). However, losses due to

64 this open N cycling have been reported to be low or limited to intermittent rain pulses in semi-
65 arid ecosystems (Evans and Burke, 2012; Jongen et al., 2013).

66

67 More intense and prolonged droughts due to climate change are expected to promote
68 open N cycling in semi-arid shrublands via sustained N processing during periods of low plant
69 uptake (Evans and Burke, 2012; Homyak et al., 2016). Invasions by exotic herbaceous species
70 have also been reported to increase soil N availability and cycling rates (Blank, 2008; Hawkes et
71 al., 2005; Wolkovich et al., 2010) by possessing traits associated with faster N turnover (Castro-
72 Díez et al., 2014). In semi-arid ecosystems this excess of N availability has been associated with
73 detrimental environmental effects, such as increasing emissions of nitrogenous greenhouse gases
74 (Homyak et al., 2016), eutrophication, fire frequency (Fenn et al., 2003), and competitive
75 advantages for exotic plant species (Laungani and Knops, 2009; Liu et al., 2018). While soil N
76 cycling alterations in response to droughts and plant invasions in semi-arid shrublands have been
77 evaluated, a mechanistic understanding of the interactive effects of these co-occurring global
78 change drivers is not only limiting our ability to model ecosystem responses to multiple global
79 change drivers but also limits improvements to conservation efforts aimed at maintaining
80 ecosystem services (Alba et al., 2017; Laungani and Knops, 2009; Valliere, 2016). Levels of
81 uncertainty arise from challenges in the evaluation of temporally dynamic abiotic and biotic
82 drivers (Alba et al., 2017) and the intrinsic variability of dryland ecosystems that are
83 characterized by distinct plant community types (Fenn et al., 2003) and hydrologic regimes
84 (D'Odorico and Bhattachan, 2012).

85

86 Here we present the results of a four-year rainfall manipulation field study in a coastal
87 sage scrub community (CSS), an understudied semi-arid Mediterranean-type, shrub-dominated
88 ecosystem distributed from coastal central California, USA to northern Baja California, Mexico,
89 that has been invaded by exotic herbaceous species (Minnich and Dezzani, 1998) and
90 experienced multi-year extreme drought conditions (Swain et al., 2018). Established exotic
91 annual grasses and forbs species differ from dominant CSS native perennial semi-deciduous
92 shrubs not only in growth form and phenology but also in distinct drought strategies
93 (Balachowski et al., 2016; Puritty et al., 2019; Wainwright et al., 2012; Wolkovich et al., 2010).
94 While CSS native shrubs are adapted to persist through seasonal droughts by partial senescence
95 of mature foliage that is released late in the dry season, exotic annual species exhibit faster
96 growth and earlier complete senescence before experiencing dehydration (Balachowski et al.,
97 2016). The monitoring of temporal variability of soil inorganic N, net N mineralization, net
98 nitrification, and NO_3^- leaching under native- and exotic-dominated rainfall manipulated plots
99 allowed us to assess interactive mechanisms of soil N alterations. Combining field manipulations
100 and functional trait approaches has become a powerful tool to improve our mechanistic
101 understanding of ecosystem responses and feedbacks to multiple global change drivers (Boyd et
102 al., 2018; Laungani and Knops, 2009). We hypothesized that the earlier senescence of exotic
103 species, and their more easily decomposable litter compared to native species, would enhance
104 soil N availability and result in more open cycling in response to droughts (Liao et al., 2008).

105

106 **Materials and Methods**

107

108 This study was conducted from 2013 – 2016 at the Santa Margarita Ecological Reserve
109 (SMER) rainfall manipulation experiment (33.44128 N, 117.1644 W), located at the Riverside-
110 San Diego county line, California, USA. The region has a semi-arid Mediterranean-type climate
111 with cool wet winters and hot dry summers with a compared 30-year average of 358 mm yr⁻¹ of
112 rainfall. The site was a grazed pasture prior to the establishment of the Reserve in 1962.
113 Currently, the area is a heterogeneous matrix of native mature shrubs such as *Artemisia*
114 *californica* and *Salvia mellifera* interspersed with exotic annual species such as *Centaurea*
115 *melitensis*, *Hirschfeldia incana*, *Erodium cicutarium* and *Bromus madritensis*. All plots were
116 located on similar soil, classified as Las Posas loam, which are well drained with an argillic
117 horizon under the top soil (<http://websoilsurvey.sc.egov.usda.gov>). Soil analyses at the
118 beginning of the experiment showed no differences in physicochemical properties between plots
119 (Table 1). We are therefore confident that the effects observed in this experiment did not arise from
120 pre-experimental variability among plots. The field experiment consisted of 30 3 m x 3 m rainfall
121 shelters covered with a clear, polycarbonate roof permitting light transmission. Half of the plots
122 were dominated by the native mature shrubs, while the remaining plots were dominated by
123 exotic annual species. To implement the rainfall manipulation, rainfall collected from the rain
124 shelters was re-applied to plots using sprinkler heads at three rainfall levels (in each vegetation
125 type, 5 replicates of 50%, 100%, and 150% of ambient rainfall) after rainfall events. During the
126 sampling period, the region experienced below average annual rainfall with the ambient rainfall
127 plots receiving 157, 125, 139, and 156 mm yr⁻¹ during water years 2013 – 2016, respectively.
128 Due to the lack of rainfall, the 50% and 100% rainfall treatments represented severe drought and
129 drought conditions, respectively, and the 150% treatment corresponded to an average water year.

130 Water year 2015 received major Pacific storms and summer rains associated with the 2015 –
131 2016 El Niño event.

132

133 *N pools*

134

135 To evaluate temporal variability in soil inorganic N, we sampled soils from all plots
136 during the winter (rainy season: October-February), spring (peak plant biomass: March-April),
137 and summer (dry season: May-September) over the 4-year sampling period, resulting in 12
138 sampling events. Soil was sampled to a depth of 15 cm using a 5 cm diameter x 15 cm deep slide
139 hammer soil core sampler. Samples were stored in a cooler for transportation and processed
140 within 24 h of collection. All visible stones, roots, and litter were removed with forceps prior to
141 analysis. Dissolved NH_4^+ and NO_3^- were determined in 0.5M potassium sulfate extracts using the
142 indophenol method and by vanadium (III) reduction, respectively, and measured on a
143 spectrophotometer (Miranda et al., 2001).

144

145 *Net N mineralization, net nitrification and leaching*

146

147 Net N mineralization and nitrification were assessed using in situ soil core incubations
148 with 4 cm diameter and 10 cm depth PVC tubes (DiStefano and Gholz, 1986). Two cores were
149 inserted into the soil inside the plots of 3 out of the 5 replicates for each plant community type
150 and each precipitation manipulation ($n_{natives}=9$ vs. $n_{exotics}=9$) at the beginning of the winter

151 seasons. One of the cores was immediately removed for analyses (T_0) and the other core, which
152 was fitted with an ion exchange resin bag at the bottom of the tube, was removed during the
153 summer season (T_F). Dissolved NH_4^+ and NO_3^- were analyzed in the potassium sulfate extracts
154 from soils in the cores and in 2 M KCl extracts from the ion exchange beads in the resin bags.
155 Annual N cycling measurements included: (1) N mineralization, calculated as the difference in
156 DIN ($NH_4^+ + NO_3^-$) concentrations between T_F and T_0 plus the DIN in resin bags, (2)
157 nitrification, calculated as the difference in NO_3^- concentrations between T_F and T_0 in resin
158 bags, and (3) leaching as the amount of DIN in resin bags (T_F). Although resin bags may not
159 retain all NH_4^+ and NO_3^- flowing down the core, the amount of DIN in resin bags provides a
160 reliable estimate of N leaching (Bhogal et al., 1999). To compliment leaching measurements,
161 immediately after the 2015 summer storms, we collected soil samples to a depth of 50 cm and
162 measured dissolved NO_3^- on water extracts using ion-chromatography (Thermo ICS-5000+).

163

164 *Statistical analyses*

165

166 Data analysis was performed in R version 3.3.3 (R Core Team, 2017). To determine the
167 effects of rainfall treatment, plant community type, season, and year and their interactions on soil
168 NH_4^+ , NO_3^- , N mineralization, nitrification, and leaching, mixed-effects repeated measures
169 analysis of variance (RM-ANOVA) was employed using the lme function within the ‘nlme’
170 package (Pinheiro et al., 2018). Linear model assumptions of normality and homoscedasticity
171 were tested using Shapiro-Wilk and Levene’s test and observed visually using Q-Q plots and
172 plotting residuals against fitted values, respectively. When significant effects were found,
173 multiple comparisons between treatments, seasons, and years were performed using the glht

174 function within the ‘multcomp’ package (Hothorn et al., 2008). P-values less than 0.05 were
175 considered significant.

176

177 **Results**

178

179 *Nitrogen pools*

180

181 We detected significant differences in soil NH_4^+ and NO_3^- concentrations between years
182 ($p_{NH_4^+} = 0.0002$ and $p_{NO_3^-} < 0.0001$), seasons ($p_{NH_4^+} = 0.001$ and $p_{NO_3^-} = 0.01$), rainfall
183 treatments ($p_{NH_4^+} = 0.001$ and $p_{NO_3^-} = 0.006$), and plant community types ($p_{NH_4^+} = 0.02$ and
184 $p_{NO_3^-} < 0.0001$; Table 1). Mean annual soil NH_4^+ and NO_3^- concentrations increased over the 4-
185 year sampling period under both plant community types, with a more pronounced increase in
186 exotic-dominated plots (Fig. 1a-b). In 2016, annual average soil NH_4^+ was 3.1 and 2.5 times
187 higher in exotic- and native-dominated plots, respectively, when compared to 2013 (Fig. 1a).
188 Annual average soil NO_3^- was 6.0 and 8.4 times higher in native- and exotic-dominated plots,
189 respectively, in 2016 when compared to 2013 (Fig. 1b). Increases in soil NH_4^+ and NO_3^-
190 concentrations were also observed during the winter season (Fig. 2a-b) and in the 50% rainfall
191 treatment under both plant communities (Fig. 3a-b). Soil NH_4^+ in the 50% treatment (severe
192 drought) was 2.0 and 1.3 times higher in exotic- and native-dominated plots, respectively, when
193 compared to the 150% treatment (Fig. 3a). Soil NO_3^- was 2.0 and 1.8 times higher in exotic- and
194 native-dominated plots, respectively, in the 50% treatment when compared to the 150% treatment
195 (Fig. 3b). Soil NH_4^+ concentrations were increased in exotic plots by approximately 43% based

196 on average values across the entire time of observations (estimated mean \pm SD, native-
197 dominated: $2.4 \pm 3.6 \mu\text{g N g}^{-1}$ soil vs. exotic-dominated: $3.4 \pm 4.2 \mu\text{g N g}^{-1}$ soil). Soil
198 NO_3^- concentrations were increased in exotic plots by approximately 219% (estimated mean \pm
199 SD, native-dominated: $2.2 \pm 3.2 \mu\text{g N g}^{-1}$ soil vs. exotic-dominated: $7.0 \pm 8.2 \mu\text{g N g}^{-1}$
200 soil).

201

202 *Net N mineralization, net nitrification and leaching*

203

204 Net N mineralization was influenced by year ($p = 0.03$), and there was a moderately
205 significant interaction between rainfall treatment and plant community type ($p = 0.07$; Table 1).
206 Annual net N mineralization was more variable in exotic-dominated plots (Fig. 1c). In exotic
207 plots, net N mineralization rates increased from 2014 to 2015 and decreased from 2015 to 2016.
208 Highest net N mineralization occurred in 2015 in exotic-dominated plots (Fig. 1c). During 2016,
209 net N mineralization was negative (i.e. net immobilization) in exotic plots (Fig. 1c). Net N
210 mineralization differed between the two community types in response to drought. In exotic-
211 dominated plots net N mineralization increased with increasing drought intensity, while in native
212 plots the opposite pattern was present with the highest levels observed in the 150% treatment
213 (Fig. 3c).

214

215 While we did not detect significant effects of rainfall treatment, plant community type, or
216 year on net nitrification (Table 2), similar patterns to net N mineralization rates were observed
217 (Figure 1d & 3d). Highest net nitrification rates occurred in 2015 in exotic-dominated plots, and

218 negative net nitrification was observed in 2016 (Fig. 1d). The response of net nitrification to
219 drought differed in direction between the two community types, although this was non-
220 significant. In exotic-dominated plots net nitrification increased with drought intensity, and in
221 native plots highest levels of net nitrification were found in the 150% treatment (Fig. 3d).

222

223 Leaching (DIN in resin bags) was significantly influenced by the interaction between
224 plant community type and year ($p < 0.0001$). In soils under native plants, leaching was higher
225 during 2015 when compared with 2013, 2014, and 2016 (Fig. 4a). In soils under exotic plants,
226 leaching was higher during 2015 and 2016 when compared with 2013 and 2014 (Fig. 4a). During
227 2015 and 2016 leaching was 1.9 and 4.1 times higher in exotic-dominated plots when compared
228 to native plots, respectively. After the 2015 summer rains we measured a 1.9, 3.9, 5.0, 6.7, and
229 16.4 times increase in soil dissolved NO_3^- concentrations in exotic plots when compared to
230 native plots at 0-10, 10-20, 20-30, 30-40, and 40-50 cm soil depths, respectively (Fig. 4b).

231

232 **Discussion**

233 *Soil inorganic N accumulation under drier conditions*

234

235 Consistent with the generalization that drier conditions facilitate inorganic N
236 accumulation (Evans and Burke, 2012; Homyak et al., 2017, 2016), in this study we found higher
237 levels of soil NH_4^+ and NO_3^- concentrations in response to increasing drought severity due both
238 to rainfall manipulations and over the 4-year period of below average annual rainfall under both

239 plant community types. We observed several patterns that provide insight into the mechanisms of
240 soil inorganic N accumulation. First, annual rates of net N mineralization and nitrification rates
241 were observed to be resistant to drought severity on average (rainfall treatments, Table 2), and
242 net nitrification did not differ significantly during the 4 consecutive years of below average
243 annual rainfall, suggesting statistically unaltered contributions to the supply of NH_4^+ and NO_3^-
244 concentrations during dry periods. The resistance of microbial processes to low water potentials
245 has been reported particularly in dryland ecosystems due to their natural exposure to seasonal
246 droughts (Canarini et al., 2017; Drigo et al., 2017). However, it is also possible that there were
247 seasonal changes in N cycling rates that were not detected with our annual measures. Increased
248 inorganic N in response to drought may have also been caused by other drought-tolerant
249 microbial N sources, such as asymbiotic N fixation, (Boring et al., 1988), or N deposition,
250 particularly dry deposition (Valliere et al., 2017). In the location of our study, N deposition
251 occurs at levels ranging from 9-11 kg N $ha^{-1}yr^{-1}$ (Fenn et al., 2010). Secondly, we observed a
252 significant increase in leaching during 2015 (wettest year) when compared to previous years
253 under both plant community types. This pattern suggests inorganic N accumulates through
254 reduced leaching losses during drier conditions. Nitrate is highly mobile, and its diffusivity has
255 been previously reported limited under drought conditions (Evans and Burke, 2012). Lastly,
256 differences in soil inorganic N concentrations and leaching between exotic- and native-
257 dominated plots suggest that plant-derived mechanisms influence N accumulation.

258

259 *Soil inorganic N accumulation as affected by invasion*

260

261 In comparison to soils under native plants, soils under exotic herbaceous species were
262 observed to have higher soil inorganic N accumulation. We propose that exotic herbaceous
263 species increased soil inorganic N availability by making N cycling more rapid. Litter N release
264 may have been faster from the exotic herbaceous species because of their higher litter quality and
265 their earlier and complete senescence of biomass. This explanation is consistent with the
266 generalization that invasive plants increase N pools by possessing traits associated with faster N
267 turnover (Castro-Díez et al., 2014; Liao et al., 2008). Additionally, invasion by exotic species
268 may accelerate N release to soils via increased litter susceptibility to UV photodegradation
269 compared with native litter (Esch et al., 2019a). Vegetation structure could also play a role,
270 aboveground cover by exotic herbaceous species when compared with native shrubs could
271 enhance the effects of solar radiation on litter decomposition. Plant traits associated with N-
272 fixation have been proposed as another mechanism by which invasive plants enhance N pools
273 (Castro-Díez et al., 2014). While not evaluated in this study, higher asymbiotic N fixation
274 activity may be a mechanism by which the non N-fixing exotic species could have increased soil
275 N inputs (Castro-Díez et al., 2014; Eisele et al., 1989; Mack et al., 2001). An increase in the
276 relative abundance of Cyanobacterial DNA sequences has been found in our exotic-dominated
277 plots (Pérez Castro et al., 2019), which are associated with N fixation (Green et al., 2008).

278

279 *The interaction of invasion and dry conditions*

280

281 Contrary to soils under native-dominated plots, we observed a moderate positive
282 relationship between drought severity and N accumulation and net transformations under exotic-
283 dominated plant communities. We proposed that accelerated N cycling by exotic herbaceous

284 species coupled with drought-induced increases in N accumulation generated a larger inorganic
285 N pool. However, significant decreases in soil NH_4^+ from 2014 to 2015 (the relatively wet year),
286 and increases in N leaching and higher deep soil NO_3^- levels during 2015 in soils under exotic
287 species when compared to soils under native species, suggest that soils under exotic plants did
288 not retain the accumulated N. We speculate that the shorter phenology of the exotic annual
289 species (Esch et al., 2019b) and reduced N uptake (demand) during water inputs increased its
290 vulnerability to leaching losses. This is consistent with the hypothesis that drought-induced soil
291 inorganic N accumulation is more vulnerable to losses upon rewetting (Evans and Burke, 2012).

292

293 **Conclusions**

294 In this experimental semi-arid shrubland, we found that plant functional traits are key
295 factors in providing mechanistic explanations of soil responses and feedbacks to environmental
296 disturbances. In comparison to soils under native woody plants, we observed that soils under
297 exotic herbaceous plants had enhanced inorganic N accumulation and leaching losses after
298 experiencing severe drought conditions. Our findings indicate that distinct morphological,
299 physiological, and phenological traits of annual herbaceous species, associated with N higher
300 inputs, faster turnover, and reduced temporal uptake, play a major role in N pools and fluxes.
301 Nitrogen alterations under severe droughts and exotic plants likely produce a larger pool of N
302 that is more vulnerable to increased leaching and may provide a competitive advantage to
303 promote exotic growth and invasiveness success in these N-limited ecosystems (Liu et al., 2018;
304 Vourlitis, 2017). While this study suggests N transformation rates as mechanisms of inorganic N
305 accumulation, future efforts that measure gross N production are needed to confirm this speculation and
306 better understand microbially mediated N-cycling processes.

307

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315

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492 **Table 1.** Soil physicochemical properties (mean) in native- and exotic-dominated plots by

493 rainfall treatment prior the beginning of the experiment.

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Plant community type	Native			Exotic			
	Rainfall treatment	50%	100%	150%	50%	100%	150%
Sand (g g^{-1})		0.3	0.3	0.3	0.2	0.3	0.3
Silt (g g^{-1})		0.5	0.5	0.6	0.6	0.5	0.6
Clay (g g^{-1})		0.2	0.2	0.2	0.2	0.2	0.2
K (mg g^{-1})		0.2	0.1	0.2	0.2	0.2	0.2
Ca (mg g^{-1})		3.1	2.6	2.9	3.0	2.8	3.1
Mg (mg g^{-1})		1.3	1.3	1.3	1.3	1.3	1.5
Al (mg g^{-1})		2.7	2.2	2.7	2.7	2.7	2.6
pH (saturation paste)		6.1	6.2	6.1	6.2	6.1	6.2
Soil organic matter (g g^{-1})		0.1	0.1	0.1	0.1	0.1	0.1

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505 **Table 2.** Summary of p-values for the linear mixed model for effects of rainfall treatments, plant
506 community type, season, year and the interaction on soil N parameters (there were no significant
507 4-way interaction).

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Factor(s)	NH4	NO3	Net Min	Net Nit	Leaching
Rainfall treatment	0.0013*	0.0059*	0.2667	0.3516	0.2499
Plant community type	0.0168*	<0.0001*	0.1316	0.2969	<0.0001*
Season	0.0006*	0.0010*	N.A	N.A	N.A
Year	0.0002*	<0.0001*	0.0265*	0.1548	<0.0001*
Rain:Plant	0.1983	0.2178	0.0698.	0.3176	0.4442
Rain:Season	0.2038	0.1680	N.A.	N.A.	N.A.
Plant:Season	0.5998	0.7013	N.A.	N.A.	N.A.
Rain:Year	0.1617	0.2553	0.7984	0.7497	0.3474
Plant:Year	0.1145	<0.0001*	0.2107	0.1816	<0.0001*
Season:Year	0.0863.	0.0029*	N.A.	N.A.	N.A.
Rain:Plant:Season	0.0365*	0.1335	N.A.	N.A.	N.A.
Rain:Plant:Year	0.7893	0.6098	0.7927	0.8729	0.5234
Rain:Season:Year	0.5423	0.5563	N.A.	N.A.	N.A.
Plant:Season:Year	0.7303	0.0523.	N.A.	N.A.	N.A.

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514 **Figure Legends**

515 **Figure 1.** Inter-annual variation of soil: (a) NH_4^+ , (b) NO_3^- , (c) N mineralization, and (d)
516 nitrification for each plant community type from 2013 to 2016 across rainfall treatments (no
517 plant community type \times rainfall treatment interaction). Yellow bars = exotic plots; green bars =
518 native plots. Error bars represent the standard error of the mean.

519 **Figure 2.** Intra-annual variation of (a) NH_4^+ and (b) NO_3^- for each plant community type from
520 winter (Wi) to summer (Su). Yellow bars = exotic plots; green bars = native plots. Error bars
521 represent the standard error of the mean.

522 **Figure 3.** Drought severity effects on (a) NH_4^+ , (b) NO_3^- , (c) N mineralization, and (d)
523 nitrification for each plant community type. Yellow bars = exotic plots; green bars = native plots.
524 Error bars represent the standard error of the mean.

525 **Figure 4.** Inter-annual variation of leaching (DIN in resin bags) and deep soil NO_3^-
526 concentrations during the 2015 summer season. Error bars represent the standard error of the
527 mean.

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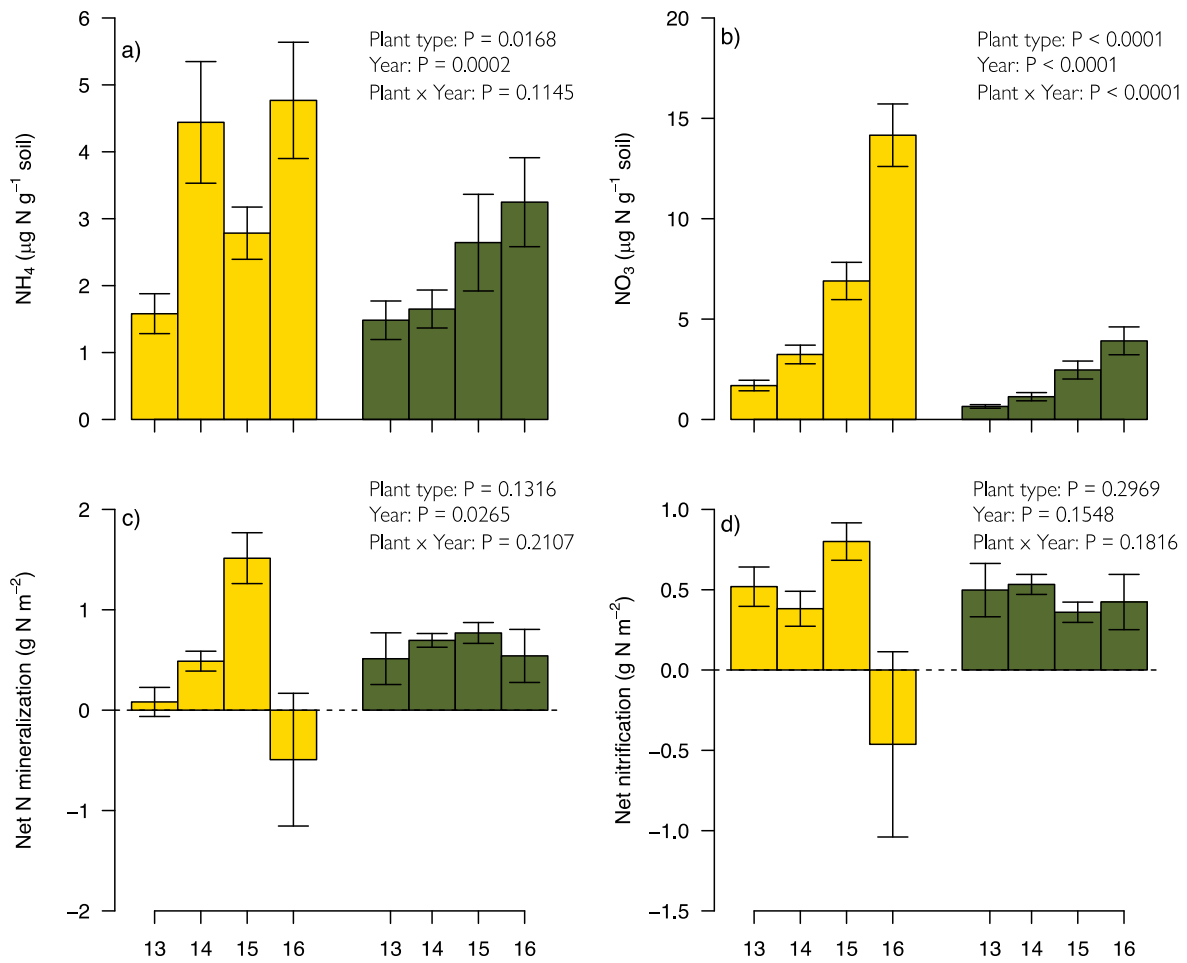
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534 **Figure 1**

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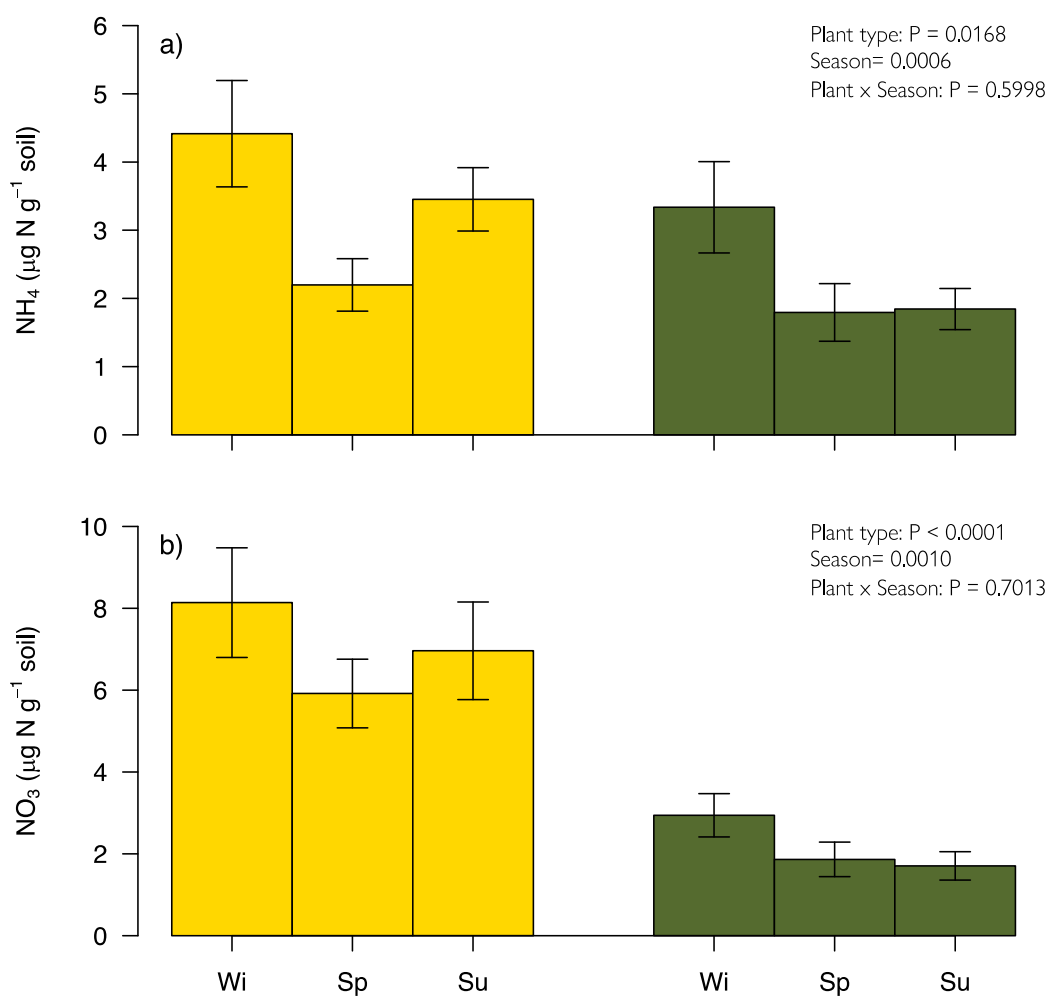
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542 **Figure 2**

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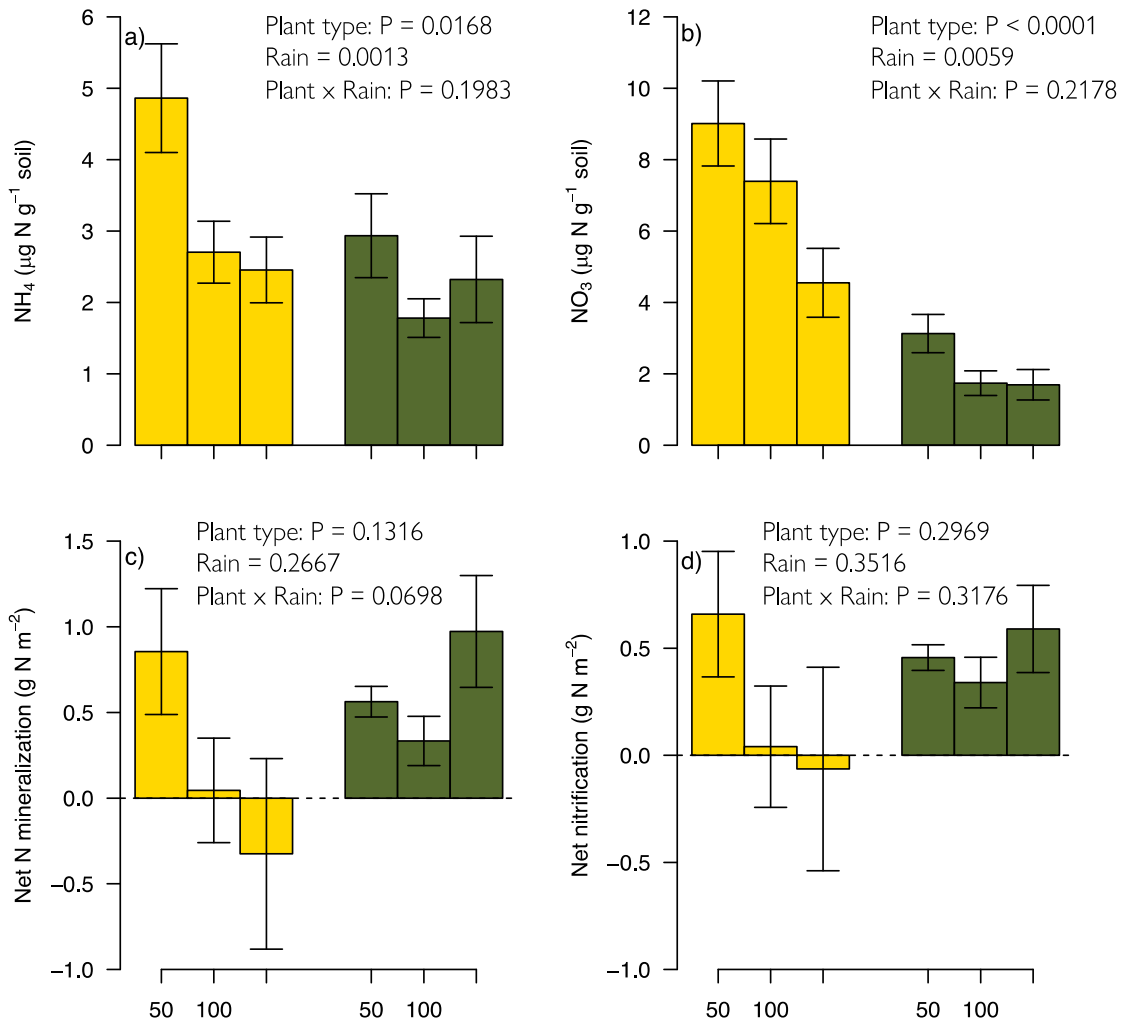
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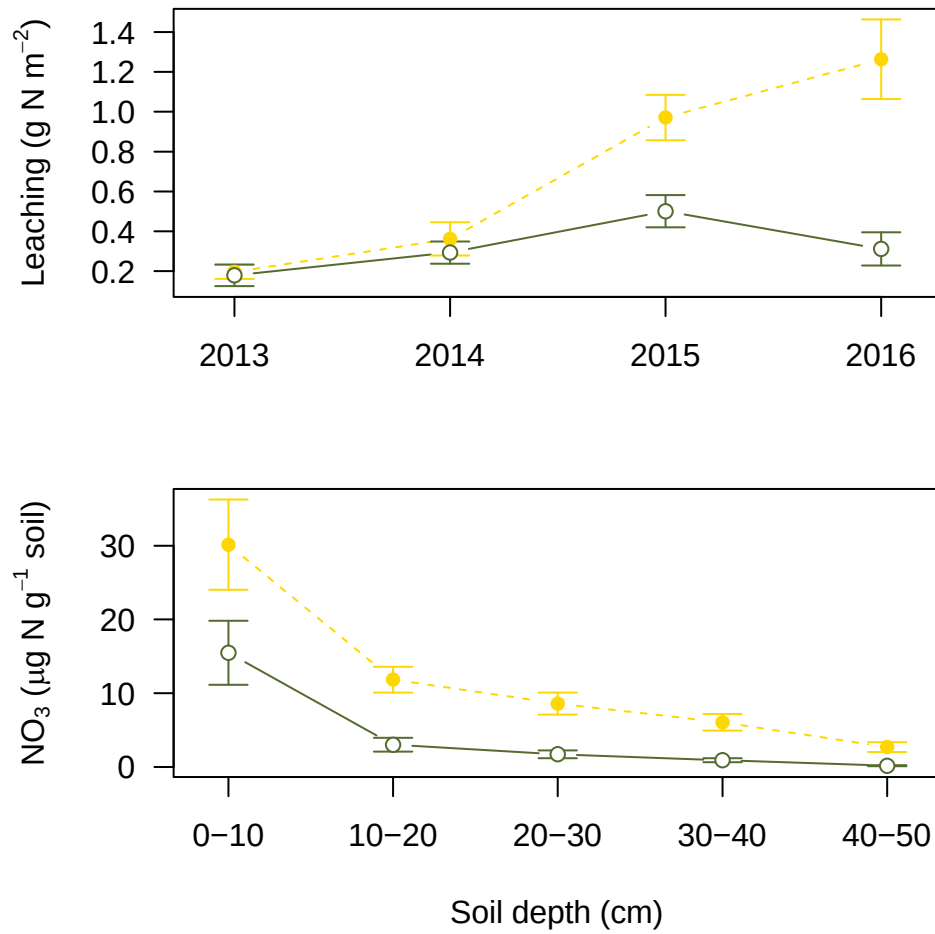
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550 **Figure 3**



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556 **Figure 4**



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Highlights

- Reduced leaching and microbial N cycling promote soil N accumulation during droughts
- Droughts and exotic plants result in the enhanced accumulation of soil N
- Exotic plants facilitate increases in N leaching upon soil rewetting

1 Title: Exotic herbaceous species interact with severe drought to alter soil N cycling in a semi-
2 arid shrubland

3

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19 **Keywords:** Climate change, Exotic species invasion, Coastal sage scrub; Soil nitrogen

20 **Abstract**

21 Mediterranean-type ecosystems are increasingly threatened by climate change and exotic annual
22 species, jeopardizing the native communities and their global biodiversity. In these systems, soil
23 nitrogen (N) limits net primary production, and its availability can be influenced by both of these
24 stressors. To understand the interactive effects of droughts and exotic herbaceous species on soil
25 N, we monitored the temporal variability of soil inorganic N, net N mineralization, net
26 nitrification, and NO_3^- leaching under native- and exotic-dominated stands exposed to rainfall
27 manipulation plots in a Mediterranean-type shrub-dominated community. Increasing drought
28 severity resulted in the accumulation of soil NH_4^+ and NO_3^- , with a more pronounced increase in
29 exotic-dominated plots. Increased net N mineralization and net nitrification and reduced leaching
30 losses were observed as mechanisms of inorganic N accumulation. In comparison to soils under
31 native plants, soils under exotic plants had enhanced leaching losses upon soil rewetting. We
32 propose that distinct traits of exotic annual herbaceous species associated with higher N inputs,
33 faster turnover, and reduced temporal uptake determine the changes in N cycling in response to
34 droughts. Severe droughts and exotic plants may produce a larger, more vulnerable pool of N
35 that is prone to losses while providing a competitive advantage to promote exotic growth in these
36 N-limited ecosystems.

37

38 **Introduction**

39

40 Nitrogen (N) cycling is being altered globally more than any other essential nutrient
41 (Fields, 2004). Agriculture and fossil fuel combustion are increasing reactive N in the

42 atmosphere (Galloway et al., 2008), droughts are increasing the ecosystems' N losses (Homyak
43 et al., 2017), and invasive plants are altering ecosystems' N pools and fluxes (Ehrenfeld, 2003;
44 Liao et al., 2008). These N alterations represent a major challenge particularly in ecosystems
45 where N limits net primary production (Delgado-Baquerizo et al., 2014), and its availability is
46 being threatened by multiple co-occurring global change drivers (Chapin et al., 2000; Sala et al.,
47 2000). For example, semi-arid shrublands are currently experiencing invasions by exotic
48 herbaceous species (Minnich and Dezzani, 1998; Yu et al., 2016), more intense and prolonged
49 droughts (Spinoni et al., 2014; Swain et al., 2018), and elevated N deposition (Valliere et al.,
50 2017), constituting multiple stressors that may have large impacts on N cycling.

51

52 In semi-arid ecosystems, N's limited accessibility is controlled by the temporal dynamics
53 of water availability (Reichmann et al., 2013; Shen et al., 2008). During wet periods, N becomes
54 accessible for microbial and plant assimilation and transformations, which contrasts to natural
55 periods of drought when decreases in diffusion and biological uptake result in the accumulation
56 of immobile N replenished from dead biomass and dry deposition (Cregger et al., 2014). The
57 synchronized immobilization by microbes and plants during wet periods coupled with limited
58 diffusivity during dry periods promotes a closed N cycle (recycling) in these systems (Homyak et
59 al., 2016; Joergensen and Wichern, 2018). However, an asynchrony/decoupling between soil N
60 availability and plant and microbial processes creates N leaks, particularly when dry soils rewet
61 outside of the plant growing season (Homyak et al., 2016). Drought-tolerant microbial
62 transformations and a lack of plant demand during ephemeral periods with mobile N availability
63 favors N losses via leaching and gaseous pathways (Dijkstra et al., 2012). However, losses due to

64 this open N cycling have been reported to be low or limited to intermittent rain pulses in semi-
65 arid ecosystems (Evans and Burke, 2012; Jongen et al., 2013).

66

67 More intense and prolonged droughts due to climate change are expected to promote
68 open N cycling in semi-arid shrublands via sustained N processing during periods of low plant
69 uptake (Evans and Burke, 2012; Homyak et al., 2016). Invasions by exotic herbaceous species
70 have also been reported to increase soil N availability and cycling rates (Blank, 2008; Hawkes et
71 al., 2005; Wolkovich et al., 2010) by possessing traits associated with faster N turnover (Castro-
72 Díez et al., 2014). In semi-arid ecosystems this excess of N availability has been associated with
73 detrimental environmental effects, such as increasing emissions of nitrogenous greenhouse gases
74 (Homyak et al., 2016), eutrophication, fire frequency (Fenn et al., 2003), and competitive
75 advantages for exotic plant species (Laungani and Knops, 2009; Liu et al., 2018). While soil N
76 cycling alterations in response to droughts and plant invasions in semi-arid shrublands have been
77 evaluated, a mechanistic understanding of the interactive effects of these co-occurring global
78 change drivers is not only limiting our ability to model ecosystem responses to multiple global
79 change drivers but also limits improvements to conservation efforts aimed at maintaining
80 ecosystem services (Alba et al., 2017; Laungani and Knops, 2009; Valliere, 2016). Levels of
81 uncertainty arise from challenges in the evaluation of temporally dynamic abiotic and biotic
82 drivers (Alba et al., 2017) and the intrinsic variability of dryland ecosystems that are
83 characterized by distinct plant community types (Fenn et al., 2003) and hydrologic regimes
84 (D'Odorico and Bhattachan, 2012).

85

86 Here we present the results of a four-year rainfall manipulation field study in a coastal
87 sage scrub community (CSS), an understudied semi-arid Mediterranean-type, shrub-dominated
88 ecosystem distributed from coastal central California, USA to northern Baja California, Mexico,
89 that has been invaded by exotic herbaceous species (Minnich and Dezzani, 1998) and
90 experienced multi-year extreme drought conditions (Swain et al., 2018). Established exotic
91 annual grasses and forbs species differ from dominant CSS native perennial semi-deciduous
92 shrubs not only in growth form and phenology but also in distinct drought strategies
93 (Balachowski et al., 2016; Puritty et al., 2019; Wainwright et al., 2012; Wolkovich et al., 2010).
94 While CSS native shrubs are adapted to persist through seasonal droughts by partial senescence
95 of mature foliage that is released late in the dry season, exotic annual species exhibit faster
96 growth and earlier complete senescence before experiencing dehydration (Balachowski et al.,
97 2016). The monitoring of temporal variability of soil inorganic N, net N mineralization, net
98 nitrification, and NO_3^- leaching under native- and exotic-dominated rainfall manipulated plots
99 allowed us to assess interactive mechanisms of soil N alterations. Combining field manipulations
100 and functional trait approaches has become a powerful tool to improve our mechanistic
101 understanding of ecosystem responses and feedbacks to multiple global change drivers (Boyd et
102 al., 2018; Laungani and Knops, 2009). We hypothesized that the earlier senescence of exotic
103 species, and their more easily decomposable litter compared to native species, would enhance
104 soil N availability and result in more open cycling in response to droughts (Liao et al., 2008).

105

106 **Materials and Methods**

107

108 This study was conducted from 2013 – 2016 at the Santa Margarita Ecological Reserve
109 (SMER) rainfall manipulation experiment (33.44128 N, 117.1644 W), located at the Riverside-
110 San Diego county line, California, USA. The region has a semi-arid Mediterranean-type climate
111 with cool wet winters and hot dry summers with a compared 30-year average of 358 mm yr⁻¹ of
112 rainfall. The site was a grazed pasture prior to the establishment of the Reserve in 1962.
113 Currently, the area is a heterogeneous matrix of native mature shrubs such as *Artemisia*
114 *californica* and *Salvia mellifera* interspersed with exotic annual species such as *Centaurea*
115 *melitensis*, *Hirschfeldia incana*, *Erodium cicutarium* and *Bromus madritensis*. All plots were
116 located on similar soil, classified as Las Posas loam, which are well drained with an argillic
117 horizon under the top soil (<http://websoilsurvey.sc.egov.usda.gov>). Soil analyses at the
118 beginning of the experiment showed no differences in physicochemical properties between plots
119 (Table 1). We are therefore confident that the effects observed in this experiment did not arise from
120 pre-experimental variability among plots. The field experiment consisted of 30 3 m x 3 m rainfall
121 shelters covered with a clear, polycarbonate roof permitting light transmission. Half of the plots
122 were dominated by the native mature shrubs, while the remaining plots were dominated by
123 exotic annual species. To implement the rainfall manipulation, rainfall collected from the rain
124 shelters was re-applied to plots using sprinkler heads at three rainfall levels (in each vegetation
125 type, 5 replicates of 50%, 100%, and 150% of ambient rainfall) after rainfall events. During the
126 sampling period, the region experienced below average annual rainfall with the ambient rainfall
127 plots receiving 157, 125, 139, and 156 mm yr⁻¹ during water years 2013 – 2016, respectively.
128 Due to the lack of rainfall, the 50% and 100% rainfall treatments represented severe drought and
129 drought conditions, respectively, and the 150% treatment corresponded to an average water year.

130 Water year 2015 received major Pacific storms and summer rains associated with the 2015 –
131 2016 El Niño event.

132

133 *N pools*

134

135 To evaluate temporal variability in soil inorganic N, we sampled soils from all plots
136 during the winter (rainy season: October-February), spring (peak plant biomass: March-April),
137 and summer (dry season: May-September) over the 4-year sampling period, resulting in 12
138 sampling events. Soil was sampled to a depth of 15 cm using a 5 cm diameter x 15 cm deep slide
139 hammer soil core sampler. Samples were stored in a cooler for transportation and processed
140 within 24 h of collection. All visible stones, roots, and litter were removed with forceps prior to
141 analysis. Dissolved NH_4^+ and NO_3^- were determined in 0.5M potassium sulfate extracts using the
142 indophenol method and by vanadium (III) reduction, respectively, and measured on a
143 spectrophotometer (Miranda et al., 2001).

144

145 *Net N mineralization, net nitrification and leaching*

146

147 Net N mineralization and nitrification were assessed using in situ soil core incubations
148 with 4 cm diameter and 10 cm depth PVC tubes (DiStefano and Gholz, 1986). Two cores were
149 inserted into the soil inside the plots of 3 out of the 5 replicates for each plant community type
150 and each precipitation manipulation ($n_{natives}=9$ vs. $n_{exotics}=9$) at the beginning of the winter

151 seasons. One of the cores was immediately removed for analyses (T_0) and the other core, which
152 was fitted with an ion exchange resin bag at the bottom of the tube, was removed during the
153 summer season (T_F). Dissolved NH_4^+ and NO_3^- were analyzed in the potassium sulfate extracts
154 from soils in the cores and in 2 M KCl extracts from the ion exchange beads in the resin bags.
155 Annual N cycling measurements included: (1) N mineralization, calculated as the difference in
156 DIN ($NH_4^+ + NO_3^-$) concentrations between T_F and T_0 plus the DIN in resin bags, (2)
157 nitrification, calculated as the difference in NO_3^- concentrations between T_F and T_0 in resin
158 bags, and (3) leaching as the amount of DIN in resin bags (T_F). Although resin bags may not
159 retain all NH_4^+ and NO_3^- flowing down the core, the amount of DIN in resin bags provides a
160 reliable estimate of N leaching (Bhogal et al., 1999). To compliment leaching measurements,
161 immediately after the 2015 summer storms, we collected soil samples to a depth of 50 cm and
162 measured dissolved NO_3^- on water extracts using ion-chromatography (Thermo ICS-5000+).

163

164 *Statistical analyses*

165

166 Data analysis was performed in R version 3.3.3 (R Core Team, 2017). To determine the
167 effects of rainfall treatment, plant community type, season, and year and their interactions on soil
168 NH_4^+ , NO_3^- , N mineralization, nitrification, and leaching, mixed-effects repeated measures
169 analysis of variance (RM-ANOVA) was employed using the lme function within the ‘nlme’
170 package (Pinheiro et al., 2018). Linear model assumptions of normality and homoscedasticity
171 were tested using Shapiro-Wilk and Levene’s test and observed visually using Q-Q plots and
172 plotting residuals against fitted values, respectively. When significant effects were found,
173 multiple comparisons between treatments, seasons, and years were performed using the glht

174 function within the ‘multcomp’ package (Hothorn et al., 2008). P-values less than 0.05 were
175 considered significant.

176

177 **Results**

178

179 *Nitrogen pools*

180

181 We detected significant differences in soil NH_4^+ and NO_3^- concentrations between years
182 ($p_{NH_4^+} = 0.0002$ and $p_{NO_3^-} < 0.0001$), seasons ($p_{NH_4^+} = 0.001$ and $p_{NO_3^-} = 0.01$), rainfall
183 treatments ($p_{NH_4^+} = 0.001$ and $p_{NO_3^-} = 0.006$), and plant community types ($p_{NH_4^+} = 0.02$ and
184 $p_{NO_3^-} < 0.0001$; Table 1). Mean annual soil NH_4^+ and NO_3^- concentrations increased over the 4-
185 year sampling period under both plant community types, with a more pronounced increase in
186 exotic-dominated plots (Fig. 1a-b). In 2016, annual average soil NH_4^+ was 3.1 and 2.5 times
187 higher in exotic- and native-dominated plots, respectively, when compared to 2013 (Fig. 1a).
188 Annual average soil NO_3^- was 6.0 and 8.4 times higher in native- and exotic-dominated plots,
189 respectively, in 2016 when compared to 2013 (Fig. 1b). Increases in soil NH_4^+ and NO_3^-
190 concentrations were also observed during the winter season (Fig. 2a-b) and in the 50% rainfall
191 treatment under both plant communities (Fig. 3a-b). Soil NH_4^+ in the 50% treatment (severe
192 drought) was 2.0 and 1.3 times higher in exotic- and native-dominated plots, respectively, when
193 compared to the 150% treatment (Fig. 3a). Soil NO_3^- was 2.0 and 1.8 times higher in exotic- and
194 native-dominated plots, respectively, in the 50% treatment when compared to the 150% treatment
195 (Fig. 3b). Soil NH_4^+ concentrations were increased in exotic plots by approximately 43% based

196 on average values across the entire time of observations (estimated mean \pm SD, native-
197 dominated: $2.4 \pm 3.6 \mu\text{g N g}^{-1}$ soil vs. exotic-dominated: $3.4 \pm 4.2 \mu\text{g N g}^{-1}$ soil). Soil
198 NO_3^- concentrations were increased in exotic plots by approximately 219% (estimated mean \pm
199 SD, native-dominated: $2.2 \pm 3.2 \mu\text{g N g}^{-1}$ soil vs. exotic-dominated: $7.0 \pm 8.2 \mu\text{g N g}^{-1}$
200 soil).

201

202 *Net N mineralization, net nitrification and leaching*

203

204 Net N mineralization was influenced by year ($p = 0.03$), and there was a moderately
205 significant interaction between rainfall treatment and plant community type ($p = 0.07$; Table 1).
206 Annual net N mineralization was more variable in exotic-dominated plots (Fig. 1c). In exotic
207 plots, net N mineralization rates increased from 2014 to 2015 and decreased from 2015 to 2016.
208 Highest net N mineralization occurred in 2015 in exotic-dominated plots (Fig. 1c). During 2016,
209 net N mineralization was negative (i.e. net immobilization) in exotic plots (Fig. 1c). Net N
210 mineralization differed between the two community types in response to drought. In exotic-
211 dominated plots net N mineralization increased with increasing drought intensity, while in native
212 plots the opposite pattern was present with the highest levels observed in the 150% treatment
213 (Fig. 3c).

214

215 While we did not detect significant effects of rainfall treatment, plant community type, or
216 year on net nitrification (Table 2), similar patterns to net N mineralization rates were observed
217 (Figure 1d & 3d). Highest net nitrification rates occurred in 2015 in exotic-dominated plots, and

218 negative net nitrification was observed in 2016 (Fig. 1d). The response of net nitrification to
219 drought differed in direction between the two community types, although this was non-
220 significant. In exotic-dominated plots net nitrification increased with drought intensity, and in
221 native plots highest levels of net nitrification were found in the 150% treatment (Fig. 3d).

222

223 Leaching (DIN in resin bags) was significantly influenced by the interaction between
224 plant community type and year ($p < 0.0001$). In soils under native plants, leaching was higher
225 during 2015 when compared with 2013, 2014, and 2016 (Fig. 4a). In soils under exotic plants,
226 leaching was higher during 2015 and 2016 when compared with 2013 and 2014 (Fig. 4a). During
227 2015 and 2016 leaching was 1.9 and 4.1 times higher in exotic-dominated plots when compared
228 to native plots, respectively. After the 2015 summer rains we measured a 1.9, 3.9, 5.0, 6.7, and
229 16.4 times increase in soil dissolved NO_3^- concentrations in exotic plots when compared to
230 native plots at 0-10, 10-20, 20-30, 30-40, and 40-50 cm soil depths, respectively (Fig. 4b).

231

232 **Discussion**

233 *Soil inorganic N accumulation under drier conditions*

234

235 Consistent with the generalization that drier conditions facilitate inorganic N
236 accumulation (Evans and Burke, 2012; Homyak et al., 2017, 2016), in this study we found higher
237 levels of soil NH_4^+ and NO_3^- concentrations in response to increasing drought severity due both
238 to rainfall manipulations and over the 4-year period of below average annual rainfall under both

239 plant community types. We observed several patterns that provide insight into the mechanisms of
240 soil inorganic N accumulation. First, annual rates of net N mineralization and nitrification rates
241 were observed to be resistant to drought severity on average (rainfall treatments, Table 2), and
242 net nitrification did not differ significantly during the 4 consecutive years of below average
243 annual rainfall, suggesting statistically unaltered contributions to the supply of NH_4^+ and NO_3^-
244 concentrations during dry periods. The resistance of microbial processes to low water potentials
245 has been reported particularly in dryland ecosystems due to their natural exposure to seasonal
246 droughts (Canarini et al., 2017; Drigo et al., 2017). However, it is also possible that there were
247 seasonal changes in N cycling rates that were not detected with our annual measures. Increased
248 inorganic N in response to drought may have also been caused by other drought-tolerant
249 microbial N sources, such as asymbiotic N fixation, (Boring et al., 1988), or N deposition,
250 particularly dry deposition (Valliere et al., 2017). In the location of our study, N deposition
251 occurs at levels ranging from 9-11 kg N $ha^{-1}yr^{-1}$ (Fenn et al., 2010). Secondly, we observed a
252 significant increase in leaching during 2015 (wettest year) when compared to previous years
253 under both plant community types. This pattern suggests inorganic N accumulates through
254 reduced leaching losses during drier conditions. Nitrate is highly mobile, and its diffusivity has
255 been previously reported limited under drought conditions (Evans and Burke, 2012). Lastly,
256 differences in soil inorganic N concentrations and leaching between exotic- and native-
257 dominated plots suggest that plant-derived mechanisms influence N accumulation.

258

259 *Soil inorganic N accumulation as affected by invasion*

260

261 In comparison to soils under native plants, soils under exotic herbaceous species were
262 observed to have higher soil inorganic N accumulation. We propose that exotic herbaceous
263 species increased soil inorganic N availability by making N cycling more rapid. Litter N release
264 may have been faster from the exotic herbaceous species because of their higher litter quality and
265 their earlier and complete senescence of biomass. This explanation is consistent with the
266 generalization that invasive plants increase N pools by possessing traits associated with faster N
267 turnover (Castro-Díez et al., 2014; Liao et al., 2008). Additionally, invasion by exotic species
268 may accelerate N release to soils via increased litter susceptibility to UV photodegradation
269 compared with native litter (Esch et al., 2019a). Vegetation structure could also play a role,
270 aboveground cover by exotic herbaceous species when compared with native shrubs could
271 enhance the effects of solar radiation on litter decomposition. Plant traits associated with N-
272 fixation have been proposed as another mechanism by which invasive plants enhance N pools
273 (Castro-Díez et al., 2014). While not evaluated in this study, higher asymbiotic N fixation
274 activity may be a mechanism by which the non N-fixing exotic species could have increased soil
275 N inputs (Castro-Díez et al., 2014; Eisele et al., 1989; Mack et al., 2001). An increase in the
276 relative abundance of Cyanobacterial DNA sequences has been found in our exotic-dominated
277 plots (Pérez Castro et al., 2019), which are associated with N fixation (Green et al., 2008).

278

279 *The interaction of invasion and dry conditions*

280

281 Contrary to soils under native-dominated plots, we observed a moderate positive
282 relationship between drought severity and N accumulation and net transformations under exotic-
283 dominated plant communities. We proposed that accelerated N cycling by exotic herbaceous

284 species coupled with drought-induced increases in N accumulation generated a larger inorganic
285 N pool. However, significant decreases in soil NH_4^+ from 2014 to 2015 (the relatively wet year),
286 and increases in N leaching and higher deep soil NO_3^- levels during 2015 in soils under exotic
287 species when compared to soils under native species, suggest that soils under exotic plants did
288 not retain the accumulated N. We speculate that the shorter phenology of the exotic annual
289 species (Esch et al., 2019b) and reduced N uptake (demand) during water inputs increased its
290 vulnerability to leaching losses. This is consistent with the hypothesis that drought-induced soil
291 inorganic N accumulation is more vulnerable to losses upon rewetting (Evans and Burke, 2012).

292

293 **Conclusions**

294 In this experimental semi-arid shrubland, we found that plant functional traits are key
295 factors in providing mechanistic explanations of soil responses and feedbacks to environmental
296 disturbances. In comparison to soils under native woody plants, we observed that soils under
297 exotic herbaceous plants had enhanced inorganic N accumulation and leaching losses after
298 experiencing severe drought conditions. Our findings indicate that distinct morphological,
299 physiological, and phenological traits of annual herbaceous species, associated with N higher
300 inputs, faster turnover, and reduced temporal uptake, play a major role in N pools and fluxes.
301 Nitrogen alterations under severe droughts and exotic plants likely produce a larger pool of N
302 that is more vulnerable to increased leaching and may provide a competitive advantage to
303 promote exotic growth and invasiveness success in these N-limited ecosystems (Liu et al., 2018;
304 Vourlitis, 2017). While this study suggests N transformation rates as mechanisms of inorganic N
305 accumulation, future efforts that measure gross N production are needed to confirm this speculation and
306 better understand microbially mediated N-cycling processes.

307

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315

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493 **Table 1.** Soil physicochemical properties (mean) in native- and exotic-dominated plots by
 494 rainfall treatment prior the beginning of the experiment.

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Plant community type Rainfall treatment	Native			Exotic		
	50%	100%	150%	50%	100%	150%
Sand (g g^{-1})	0.3	0.3	0.3	0.2	0.3	0.3
Silt (g g^{-1})	0.5	0.5	0.6	0.6	0.5	0.6
Clay (g g^{-1})	0.2	0.2	0.2	0.2	0.2	0.2
K (mg g^{-1})	0.2	0.1	0.2	0.2	0.2	0.2
Ca (mg g^{-1})	3.1	2.6	2.9	3.0	2.8	3.1
Mg (mg g^{-1})	1.3	1.3	1.3	1.3	1.3	1.5
Al (mg g^{-1})	2.7	2.2	2.7	2.7	2.7	2.6
pH (saturation paste)	6.1	6.2	6.1	6.2	6.1	6.2
Soil organic matter (g g^{-1})	0.1	0.1	0.1	0.1	0.1	0.1

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506 **Table 2.** Summary of p-values for the linear mixed model for effects of rainfall treatments, plant
 507 community type, season, year and the interaction on soil N parameters (there were no significant
 508 4-way interaction).

509

Factor(s)	NH4	NO3	Net Min	Net Nit	Leaching
Rainfall treatment	0.0013*	0.0059*	0.2667	0.3516	0.2499
Plant community type	0.0168*	<0.0001*	0.1316	0.2969	<0.0001*
Season	0.0006*	0.0010*	N.A	N.A	N.A
Year	0.0002*	<0.0001*	0.0265*	0.1548	<0.0001*
Rain:Plant	0.1983	0.2178	0.0698.	0.3176	0.4442
Rain:Season	0.2038	0.1680	N.A.	N.A.	N.A.
Plant:Season	0.5998	0.7013	N.A.	N.A.	N.A.
Rain:Year	0.1617	0.2553	0.7984	0.7497	0.3474
Plant:Year	0.1145	<0.0001*	0.2107	0.1816	<0.0001*
Season:Year	0.0863.	0.0029*	N.A.	N.A.	N.A.
Rain:Plant:Season	0.0365*	0.1335	N.A.	N.A.	N.A.
Rain:Plant:Year	0.7893	0.6098	0.7927	0.8729	0.5234
Rain:Season:Year	0.5423	0.5563	N.A.	N.A.	N.A.
Plant:Season:Year	0.7303	0.0523.	N.A.	N.A.	N.A.

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515 **Figure Legends**

516 **Figure 1.** Inter-annual variation of soil: (a) NH_4^+ , (b) NO_3^- , (c) N mineralization, and (d)
517 nitrification for each plant community type from 2013 to 2016 across rainfall treatments (no
518 plant community type \times rainfall treatment interaction). Yellow bars = exotic plots; green bars =
519 native plots. Error bars represent the standard error of the mean.

520 **Figure 2.** Intra-annual variation of (a) NH_4^+ and (b) NO_3^- for each plant community type from
521 winter (Wi) to summer (Su). Yellow bars = exotic plots; green bars = native plots. Error bars
522 represent the standard error of the mean.

523 **Figure 3.** Drought severity effects on (a) NH_4^+ , (b) NO_3^- , (c) N mineralization, and (d)
524 nitrification for each plant community type. Yellow bars = exotic plots; green bars = native plots.
525 Error bars represent the standard error of the mean.

526 **Figure 4.** Inter-annual variation of leaching (DIN in resin bags) and deep soil NO_3^-
527 concentrations during the 2015 summer season. Error bars represent the standard error of the
528 mean.

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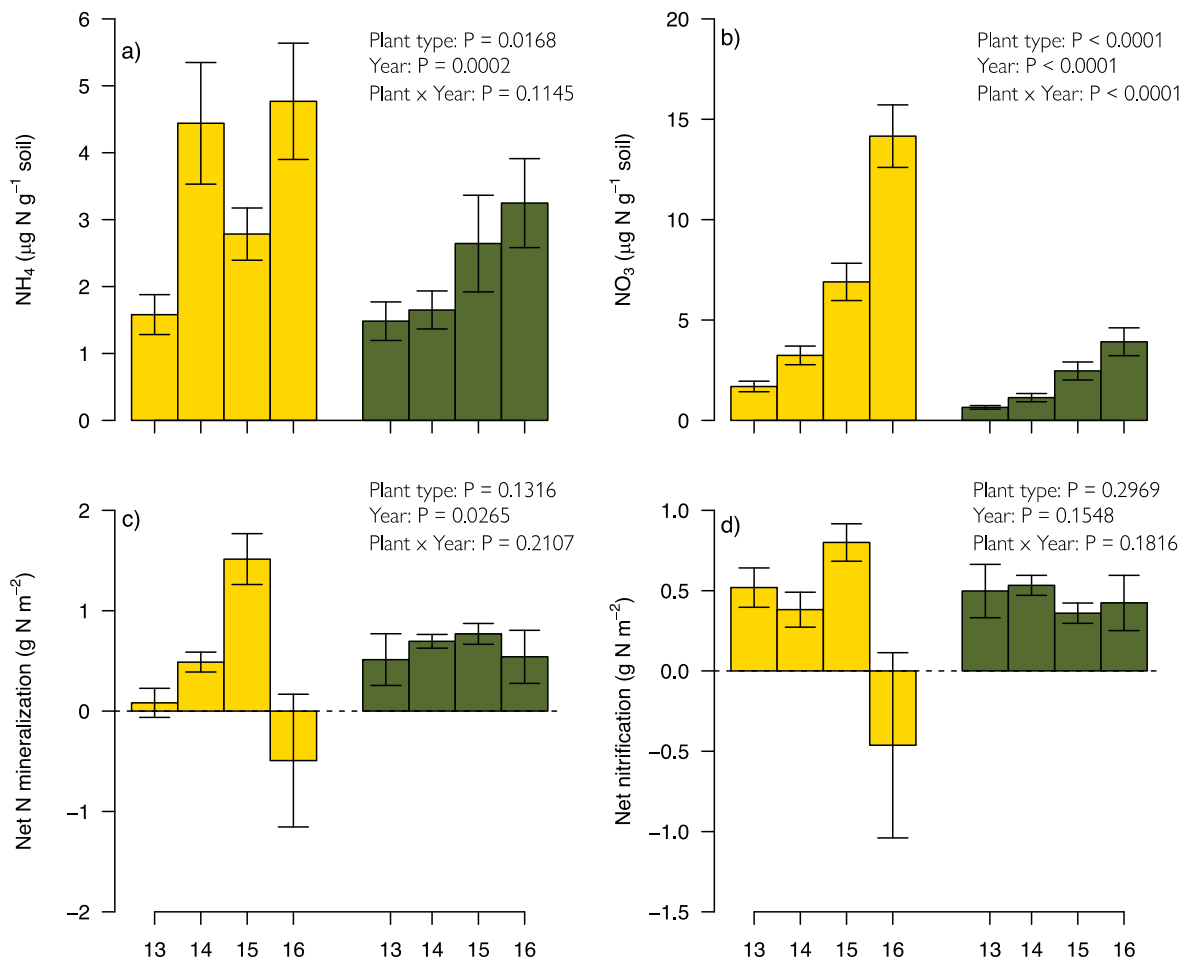
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535 **Figure 1**

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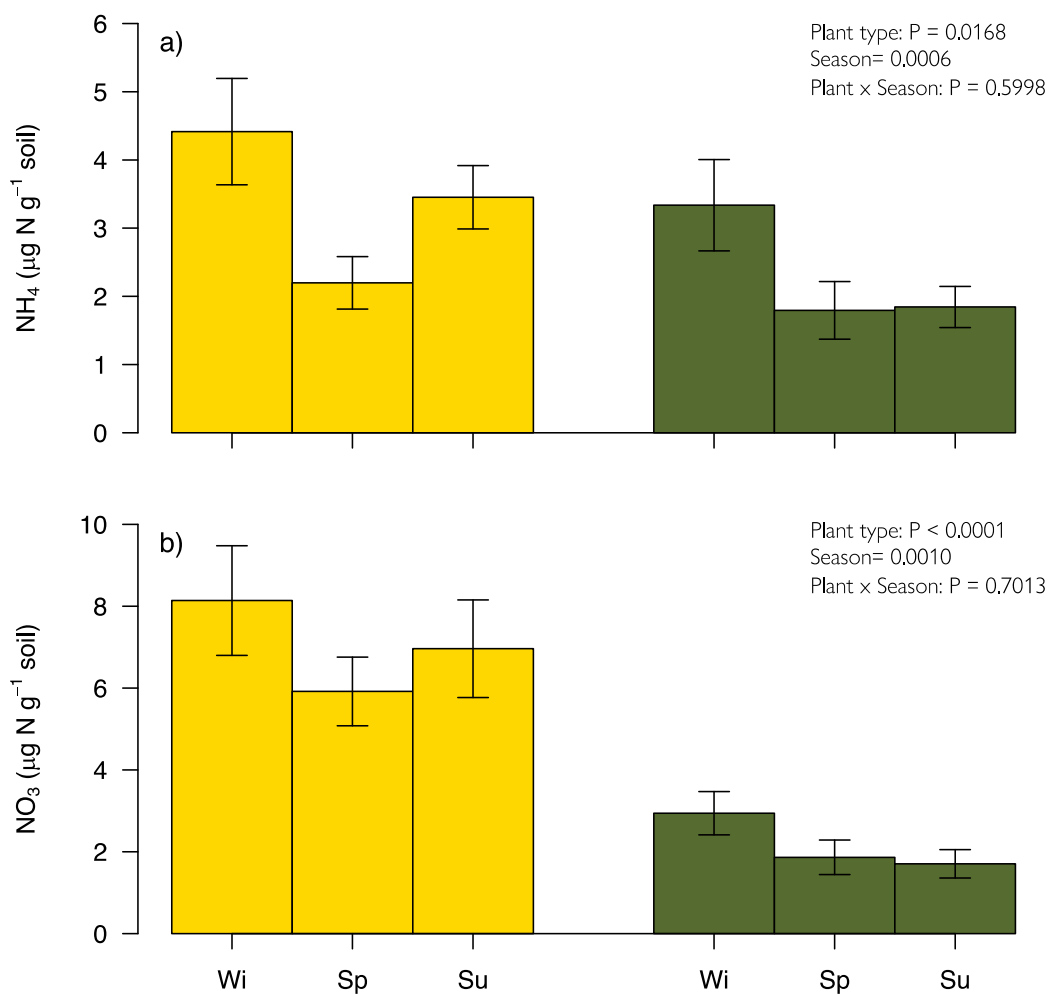
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543 **Figure 2**

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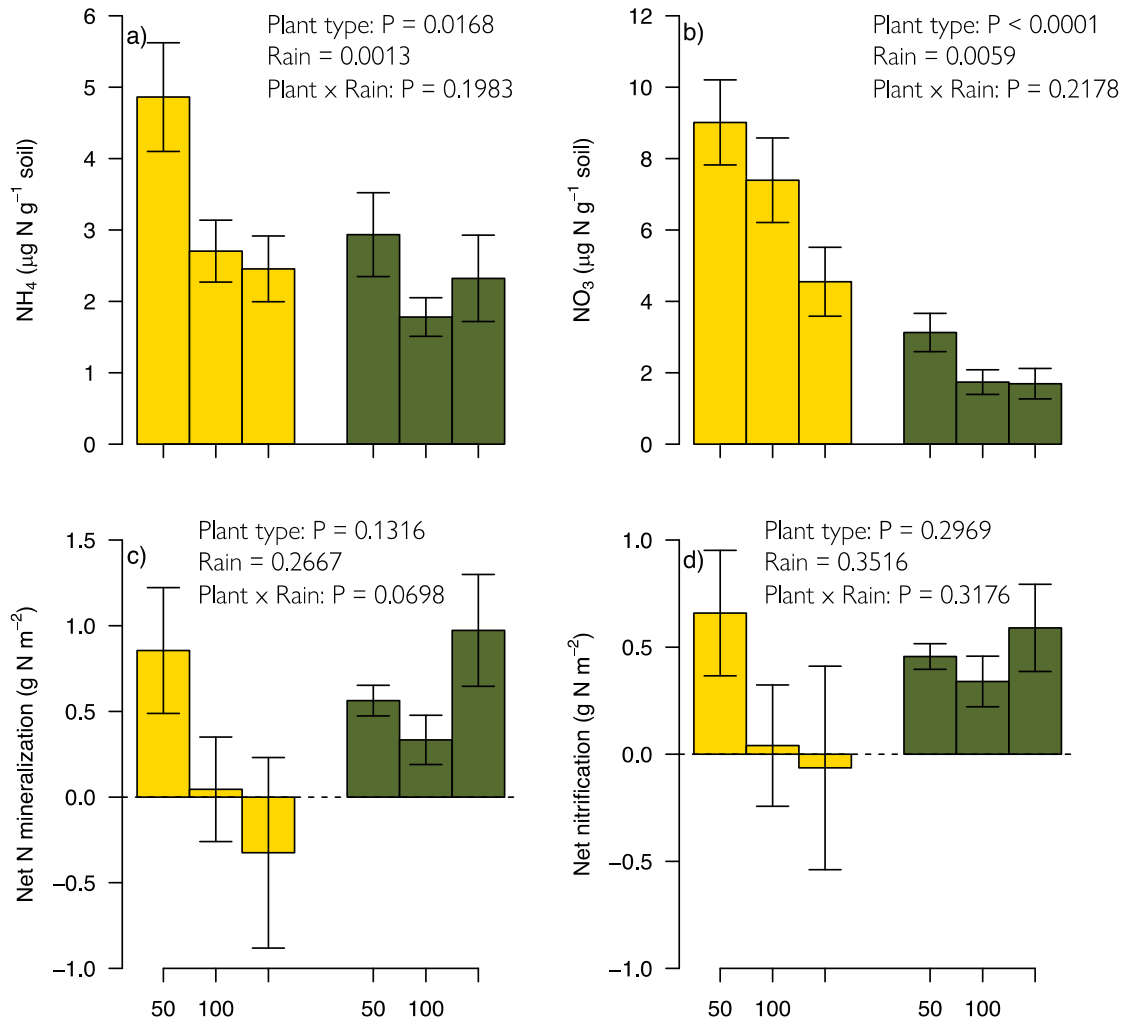
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551 **Figure 3**



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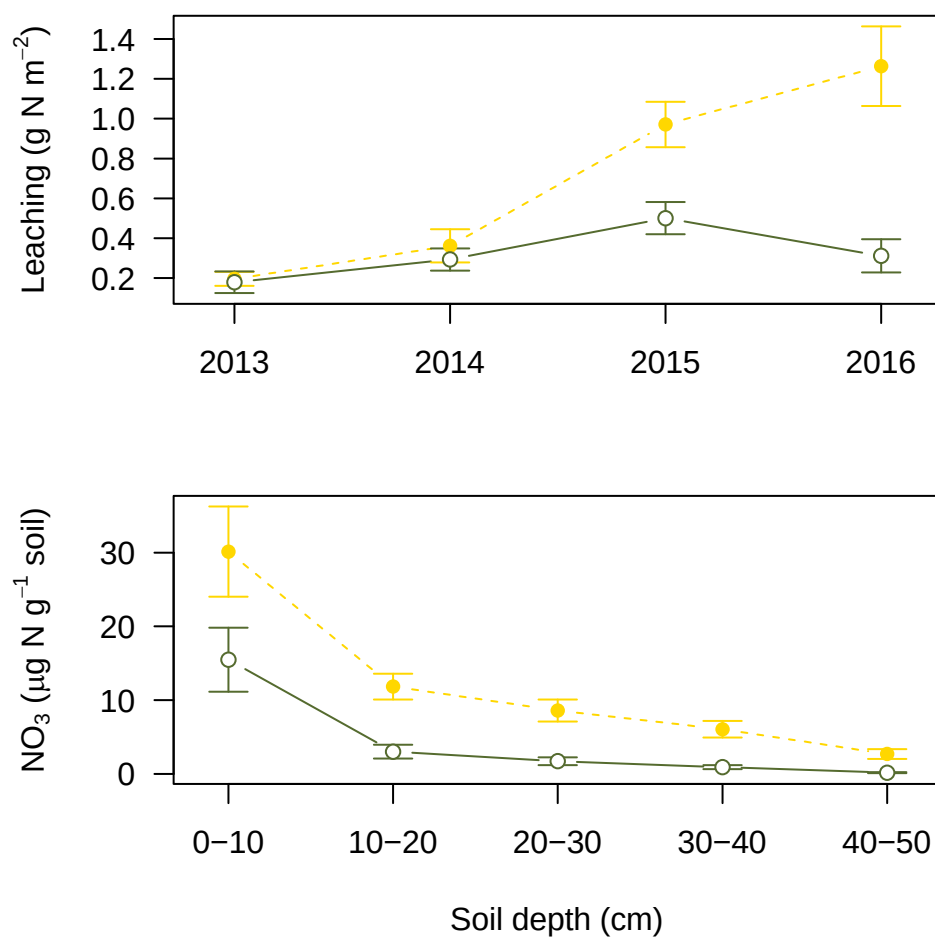
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557 **Figure 4**



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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: