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1   **Effects of drought manipulation on soil nitrogen cycling: A meta-analysis**

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Key points:

17   1. A meta-analytical approach was used on 37 studies that reduced precipitation.
18   2. The supply of mineral N did not decrease under drought treatment; extractable NH_4^+

19   increased 25%.
20   3. Microbial biomass and N_2O emissions declined and NH_4^+ increased with increasing

21   drought intensity.

Plain language summary:

Many regions on Earth are expected to become drier with climate change, which may impact N cycling rates and availability. We summarized the results of field experiments that reduced precipitation and measured the effects on N cycling. Because microbes are sensitive to changes in moisture and their activity decreases as soils dry, reducing precipitation could lower rates of N cycling. However, we found that reducing precipitation did not shut down the supply of mineral N to ecosystems and that microbial biomass increased at lower levels of proportional precipitation reduction while decreasing as water was withheld. Our results expand on early laboratory studies evaluating N dynamics in dry soils. While it is clear that microbial N transformations decrease in dry soils incubated in the laboratory, we show that under field conditions, some N cycling processes are less sensitive to precipitation reduction. Non-rainfall water inputs, such as the movement of atmospheric water vapor into soil, can stimulate drought-tolerant microbial processes in the field, and may maintain active microbial N processing despite low water content when soils are measured in bulk. Sustained N processing during drought could result in greater ecosystem N losses during subsequent wetting events.

39**Abstract**

40   Many regions on Earth are expected to become drier with climate change, which may

41impact N cycling rates and availability. We used a meta-analytical approach on the results of

42field experiments that reduced precipitation and measured N supply (i.e., indices of N

43mineralization), soil microbial biomass, inorganic N pools [ammonium (NH_4^+) and nitrate

44(NO_3^-)], and N_2O emissions. We hypothesized that N supply and N_2O emissions would be

45relatively insensitive to precipitation reduction and that reducing precipitation would increase

46extractable NH_4^+ and NO_3^- concentrations because microbial processes continue whereas plant N

47uptake diminishes with drought. In support of this hypothesis, extractable NH_4^+ increased by

4825% overall with precipitation reduction; NH_4^+ also increased significantly with increasing

49magnitude of precipitation reduction. In contrast, N supply and extractable NO_3^- did not change

50and N_2O emissions decreased with reduced precipitation. Across studies microbial biomass

51appeared unchanged, yet from the diversity of studies it was clear that proportionally smaller

52precipitation reductions increased microbial biomass, whereas larger proportional reductions in

53rainfall reduced microbial biomass; there was a positive intercept ($P = 0.005$) and a significant

54negative slope ($P = 0.0002$) for the regression of microbial biomass vs. % precipitation reduction

55(LnR = -0.009 × (% precipitation reduction) + 0.4021). Our analyses imply that relative to other

56N variables, N supply is less sensitive to reduced precipitation whereas processes producing N_2O

57decline. Drought intensity and duration, through sustained N supply, may control how much N

58becomes vulnerable to loss via hydrologic and gaseous pathways upon rewetting dry soils.

59 **1. Introduction**

60   Nitrogen (N) is an essential nutrient; it often constrains ecosystem productivity and can

61 influence rates of decomposition and carbon (C) sequestration [*LeBauer and Treseder*, 2008;

62 *Melillo et al.*, 1982; *Vitousek and Howarth*, 1991]. Understanding which environmental factors

63 govern N availability is, therefore, critical to predicting ecosystem C inputs and losses [e.g.,

64 *Averill and Waring, In press; Terrer et al.*, 2016]. Climate models predict that many regions on

65 Earth will become drier by 2100 [*Feng and Fu*, 2013; *Huang et al.*, 2016; *Maestre et al.*, 2016],

66 potentially altering rates of soil N cycling and N availability. Drought can influence N cycling

67 because water availability i) maintains hydration for the microbial processes that fix and

68 transform N and ii) controls substrate advection, diffusion, and microbial and plant access to N

69 [*Farooq et al.*, 2009; *Manzoni et al.*, 2012; *Stark and Firestone*, 1995]. These moisture effects

70 suggest that arid conditions should limit soil N cycling rates as confirmed in laboratory studies

71 [*Campbell and Biederbeck*, 1972; *Pilbeam et al.*, 1993; *Reichman et al.*, 1966]. However, under

72 *in-situ* field conditions, N fluxes and pools can increase as soils dry [*Jackson et al.*, 1988; *Parker*

73 *and Schimel*, 2011; *Sullivan et al.*, 2012].

74   Microbes respond to lower water potentials through changes in physiology and

75 community structure [*Schimel et al.*, 2007], both of which can alter rates of N cycling and

76 availability [*Fierer and Schimel*, 2002; *Fuchslueger et al.*, 2014b]. For instance, nitrifier activity

77 decreases with decreasing water potential [*Reichman et al.*, 1966; *Wetselaar*, 1968] along with

78 fluxes of nitrogen gas (N_2) and nitrous oxide (N_2O) from denitrification [*Soper et al.*, 2016a;

79 *Soper et al.*, 2016b]. Yet in the field N mineralization can continue as soils dry [*Fisher et al.*,

80 1987; *Homyak et al.*, 2016; *Parker and Schimel*, 2011; *Reichmann et al.*, 2013]. Potential

81denitrification enzyme activity can increase [*Parker and Schimel,* 2011], and extractable

82ammonium (NH_4^+) builds up [*Homyak et al.,* 2014; *Parker and Schimel,* 2011], perhaps because

83some microbes are drought-tolerant or because low water potentials can kill microbes and can

84limit plant N uptake, thereby reducing competition against surviving microbes for N [*Smith et*

85*al.,* 1997; *Zhong et al.,* 2014].

86   In support of these field observations, Sullivan et al. [2012] measured either higher or

87comparable nitrification potentials (an index of the size of the nitrifying community) in dry

88relative to wet soils across a chronosequence in Arizona, with similar observations reported from

89other dry lands [*Fisher et al.,* 1987; *Homyak et al.,* 2014; *Parker and Schimel,* 2011]. In

90synthesizing these observations, Sullivan et al. [2012] proposed that microbial processes and

91rates of N cycling must be tolerant of dry conditions, and that the size of the microbial biomass

92pool can increase as soils dry. Although a number of field studies from Mediterranean regions

93support the hypothesis that soil microbial biomass can increase as soils dry [*Boot et al.,* 2013;

94*Homyak et al.,* 2014; *Jackson et al.,* 1988; *Parker and Schimel,* 2011; *Schaeffer et al.,* 2017], it is

95unclear whether this response is exclusive to Mediterranean climates, where seasonal drought

96may select for microbes adapted to low water potentials [e.g., *Fuchslueger et al.,* 2014b; *Schimel*

97*et al.,* 2007; *Yuste et al.,* 2011]. It is also unclear whether increasing microbial biomass can

98sustain N fluxes as soils dry *in-situ*, or whether N fluxes decline in drying soils across

99ecosystems.

100   Ecosystem rainfall manipulations provide a powerful approach for understanding how

101drought affects N dynamics under *in-situ* field conditions. However, individual precipitation

102manipulations report conflicting responses of N fluxes and soil N pools [e.g., *Borken and*

103*Matzner,* 2009; *Shi et al.,* 2012; *Yahdjian et al.,* 2006]. Therefore, we addressed the following

104questions using data synthesis techniques: i) Are soil N processes and pools sensitive to drought

105across different biomes? and ii) How does reducing precipitation affect the size of the microbial

106biomass pool across ecosystems?

107   We used random-effects categorical and continuous meta-analyses to answer these

108questions across biomes with field studies that used rain-out shelters to reduce or exclude

109precipitation from plots. We identified studies that measured rates of N supply (i.e., indices of N

110mineralization), soil microbial biomass, inorganic N pools (NH_4^+ and nitrate (NO_3^-)), or N_2O

111emissions in response to precipitation reduction. We hypothesized that i) rates of N supply and

112N_2O emissions would be relatively less sensitive to precipitation reduction in the field due to

113greater microbial biomass (Fig. 1A) and higher NO_3^- concentrations that stimulate denitrification,

114and ii) field soil NH_4^+ and NO_3^- concentrations should increase under precipitation reduction due

115to ongoing microbial activity along with reduced plant growth and N uptake [Fig. 1A; *Shi et al.*,

1162014; *Wu et al.*, 2011; *Zhong et al.*, 2014].

117**2. Methods**

118*2.1. Data selection*

119   We performed meta-analyses on field studies that used rain-out shelters (structures that

120intercept precipitation) to reduce precipitation and lower soil moisture. We identified candidate

121studies by searching ISI Web of Science (Thomson Reuters, New York, NY, USA) and Google

122Scholar (Google, Mountain View, CA, USA) for published literature using a list of key words

123and wildcards (Table S1) and identified additional studies by cross-referencing. Studies were

124included if i) the authors measured soil microbial biomass, indices of N mineralization (net,

125gross, anaerobic and potential N mineralization), soil extractable NH_4^+ and NO_3^-, or nitrous oxide

126(N$_2$O) emissions; ii) the variables of interest were measured under reduced precipitation

127treatment and compared to a control; iii) experimental plots were not manipulated beyond

128precipitation reductions; and iv) means, standard deviations, and sample size were reported or

129could be determined. We were focused on understanding how drought influences rates of N

130cycling under field conditions, and we excluded greenhouse and laboratory studies. Published

131studies were collected for analysis until 1 January 2017. Overall, we found 37 studies that met

132our criteria, representing an "intermediate" sample size for meta-analysis [37 studies; *Hedges et*

133*al.,* 1999] and allowing us to analyze the best available data from ecosystem precipitation

134manipulations.

135   To maximize the likelihood of measuring a treatment effect, and meet the assumption of

136sample independence [*Gurevitch and Hedges,* 1999], we restricted our analyses to the final

137sampling date in studies that reported time series for the variables of interest—a standard meta-

138analytical approach that also minimized the likelihood of measuring disturbance effects

139associated with erecting shelters. We also refrained from using time series because small sample

140sizes limited our ability to test hypotheses concerning temporal trends and may have introduced

141biases by over-weighting studies with longer records. Moreover, if more than one paper reported

142data from the same field plots, we used the most recent or most comprehensive paper. For the

143two studies reporting data from both O and A soil horizons, we used A horizons to maintain

144consistency across studies and best represent processes occurring in the solum rather than in litter

145under various stages of decomposition. When studies reported on more than one mineral horizon,

146we used the uppermost horizon. For studies testing several levels of precipitation reduction, we

147focused on the most extreme reduction to maximize our ability to detect a rainfall exclusion

148effect. When a study included more than one site, we included as many sites as could reasonably

149 be considered independent based on geographical position, ecosystem type, or plant cover

150 [*Treseder*, 2008].

151 *2.2. Data collection*

152   We extracted means, standard deviations (SD), and sample sizes (n) for each variable of

153 interest. Data presented in figures were digitized using Plot Digitizer 2.6.6

154 (http://plotdigitizer.sourceforge.net). We grouped studies into agricultural, forest, grassland,

155 meadow, shrubland, or tropical categories. The meadow biome was used for high-elevation

156 studies at sites dominated by herbaceous plants (i.e., > 1,850 m a.s.l.) and the tropical biome for

157 subtropical, montane, humid, and old-growth tropical forests. For each study, we recorded the

158 mean annual precipitation (MAP), length of the experimental manipulation (years), %

159 precipitation reduction (100×rainfall excluded/annual precipitation), % soil moisture reduction

160 [100×(soil moisture control-soil moisture treatment)/soil moisture control)], whether the climate

161 was Mediterranean (i.e., consisting of hot dry summers and cool wet winters typical of

162 Mediterranean regions), and the type of shelter used to reduce precipitation (i.e., exclusion vs.

163 reduction). Exclusion shelters removed 100% of incoming precipitation, whereas reduction

164 shelters reduced precipitation by some fraction (i.e., < 100%) typically through openings in the

165 roof. Standard errors (SE) were converted to SDs [SD = SE ($n^{1/2}$)].

166   The studies we included measured microbial biomass by chloroform ($CHCl_3$) fumigation

167 [*Brookes et al.*, 1985], phospholipid fatty acid (PLFA) extraction [*Frostegard and Baath*, 1996],

168 or substrate induced respiration (SIR) [*Anderson and Domsch*, 1978]. There is a paucity of

169 studies measuring indices of N mineralization (net, gross, anaerobic and potential N

170 mineralization) in precipitation exclusion experiments. Because we were interested in

171 understanding whether N supply would be sensitive to reduced precipitation—not in quantifying

172the actual flux rates—we included studies measuring net N mineralization, gross N

173mineralization, anaerobic N mineralization, and potential N mineralization measurements as an

174index of N supply in control vs. rainfall exclusion plots. Only 9 studies measured nitrification,

175and we were unable to include this process in our meta-analysis. Extractable NH_4^+ and NO_3^- were

176measured after extraction in salt solution (e.g., K_2SO_4 or KCl) or in deionized water. Soil N_2O

177emissions were measured by chamber methodology [*Parkin and Venterea*, 2010].

178*2.3. Statistics*

179   We used meta-analyses to determine the effect of reduced precipitation on soil microbial

180biomass, N supply, extractable NH_4^+ and NO_3^-, and N_2O emissions. For each study and response

181variable we calculated the effect size as the natural logarithm of the response ratio (LnR) or as

182Hedge's *d*. LnR was used in the analysis of microbial biomass and extractable NH_4^+ and NO_3^-

183pools, and is defined as:

184    $$LnR = \ln\left(\frac{X_T}{X_C}\right)$$

185 where X_T is the treatment mean and X_C the control mean. If reducing precipitation increases the

186treatment mean over the control, then LnR>0. Alternatively, LnR<0 when reducing precipitation

187reduces the treatment mean relative to the control; LnR=0 when there is no effect of reducing

188precipitation.

189   LnR cannot be used for negative values—it is undefined—so we used Hedge's *d* as the

190response variable for both N supply and N_2O emissions, which both included negative means.

191Hedge's *d* is defined as:

192
$$d = \frac{(X_T - X_C)}{S} J$$

193 where S is the pooled standard deviation and J corrects for small sample bias [*Gurevitch et al.*,

194 2001]. As with LnR, *d*>0 if reducing precipitation increases the treatment mean relative to the

195 control, *d*<0 if reducing precipitation reduces the treatment mean relative to the control, and *d*=0

196 if reducing precipitation has no effect. Along with mean effect sizes, we also calculated the

197 variance (V) for both LnR and Hedge's *d* using the means, SDs, and sample sizes for treated and

198 control plots [*Gurevitch et al.*, 2001].

199   To determine whether reducing precipitation affected soil microbial biomass, N supply,

200 extractable NH_4^+ and NO_3^- pools, and N_2O emissions, we used a random effects model in the

201 MetaWin software [*Rosenberg et al.*, 2000] with a 95% confidence interval (CI) to assess

202 significant effects; CIs not overlapping with 0 indicated significant responses at $\alpha < 0.05$. We

203 used random effects categorical models to assess whether the effects of reducing precipitation on

204 the variables of interest varied by manipulation type (i.e., precipitation exclusion vs. reduction)

205 or biome (i.e., agricultural, forest, grassland, meadow, shrubland, and tropical) or whether the

206 effect of reducing precipitation on microbial biomass was exclusive to a Mediterranean climate.

207 We used a continuous random effects model between the mean effect size and MAP (a proxy of

208 overall site wetness) to test ($\alpha = 0.05$) whether wetter sites responded differently to reduced

209 precipitation than drier sites. Lastly, we also used continuous random effects models to test for

210 significant ($\alpha = 0.05$) relationships between the mean effect size and manipulation length, %

211 precipitation reduction, and % soil moisture reduction.

212   Because we used random-effects meta-analyses, we did not test for total heterogeneity

213among studies (Q_T), as substantial variation among the population of studies included in our

214analyses is assumed and is already accounted for in random-effects models [*Rosenberg*, 2013].

215For categorical meta-analyses, however, we partitioned Q_T into the amount of heterogeneity

216explained by the model structure (Q_M; between-class heterogeneity) and the amount of

217heterogeneity left unexplained after considering the model—the residual error heterogeneity (Q_E;

218within-class heterogeneity). The significance of both Q_M and Q_E was tested ($\alpha = 0.05$) against a χ^2

219distribution to determine whether the structural models could explain the total heterogeneity—

220i.e., a significant Q_M—and whether substantial heterogeneity was left unaccounted for by the

221models—i.e., a significant Q_E even if Q_M is significant [*Rosenberg*, 2013]. Lastly, we tested for

222publication bias (i.e., the likelihood of a study with high effect sizes being published over one

223with lower effect sizes) using Kendall's tau and Spearman rank correlation tests [*Holden and*

224*Treseder*, 2013].

225**3. Results**

226   We identified 37 published studies that used rain-out shelters to reduce or exclude

227precipitation across 6 biomes (agricultural, forest, grassland, meadow, shrubland, and tropical),

228from which we extracted 106 independent observations—27 observations of soil microbial

229biomass (19 $CHCl_3$ fumigation, 4 PLFA extraction, and 4 SIR), 24 of extractable NH_4^+, 24 of

230extractable NO_3^-, 16 of N supply (7 net N mineralization, 4 gross N mineralization, 3 potential N

231mineralization, and 2 anaerobic N mineralization), and 15 of N_2O emissions (Table S3). Mean

232effect sizes were not related to % soil moisture reduction ($P > 0.2$; Figs. S1-S5) and did not vary

233as a function of overall site wetness ($P > 0.08$; Fig. S6).

234*3.1. Soil microbial biomass*

235 Opposite to our predictions, reducing precipitation had no overall effect on the size of the

236soil microbial biomass pool (Fig. 2; LnR = -0.03 ± 0.17 95% CI), but the microbial biomass pool

237decreased as a function of % precipitation reduction (Fig. 2; LnR = -0.009 × (% precipitation

238reduction) + 0.4021; *P* = 0.0002). Reducing precipitation by relatively small fractions

239significantly increased the size of the microbial biomass pool (LnR intercept = 0.4021; *P* =

2400.005), whereas reducing precipitation by larger fractions reduced microbial biomass to levels

241lower than the experimental controls for each study (Fig. 2). There was no relationship between

242microbial biomass and manipulation length (Fig. S1; *P* = 0.3).

243 Based on categorical models, the response of microbial biomass to precipitation reduction

244did not depend on shelter type or Mediterranean climate ($P \geq 0.75$; Table 1), but the effect of

245biome was marginally significant (*P* = 0.065). Extraction type accounted for a large fraction of

246the total heterogeneity among studies (Q_M *P* = 0.0003) but there were no statistically significant

247differences in effect size between the extraction methods (*P* > 0.05; Table 1)—i.e., extraction

248type did not influence the response of microbial biomass to reduced precipitation.

249*3.2. N supply*

250 Consistent with our hypothesis, N supply was not affected by precipitation reduction

251(Fig. 3; Hedge's *d* = 0.03 ± 0.46 95% CI) and was not related to manipulation length or %

252precipitation reduction (Fig. S2; *P* > 0.3). Accounting for shelter type in categorical models did

253not explain much of the heterogeneity among studies (Table 1; Q_M *P* > 0.4) whereas accounting

254for biome did (Table 1; Q_M *P* = 0.035); however, differences in the effect size of N supply among

255biomes were not significant (Table 1)—i.e., differences in biomes did not influence the response

256of N supply to reduced precipitation.

257   Kendall's tau and Spearman rank correlation tests for publication bias suggest that, across

258experiments, reduced rates of N supply were more likely to be published than increased rates

259(Table S2).

260*3.3. Extractable NH_4^+ and NO_3^-*

261   As hypothesized, reducing precipitation increased extractable NH_4^+ concentrations by

26225% across studies (Fig. 4; LnR = 0.22 ± 0.21 95% CI) and these concentrations increased with

263greater % precipitation reduction (Fig. 4; LnR = 0.0142 × (% precipitation reduction) - 0.4208; *P*

264= 0.0003). In contrast to microbial biomass, reducing precipitation by relatively small fractions

265significantly decreased the size of the extractable NH_4^+ pool (Fig. 4; LnR intercept = -0.4208; *P*

266= 0.03), whereas reducing precipitation by larger fractions increased NH_4^+. Extractable NH_4^+

267concentrations were not related to manipulation length (Fig. S3; *P* = 0.2). Accounting for shelter

268type or biome in categorical models did not explain a significant fraction of heterogeneity among

269studies (Table 1; Q_M *P* > 0.7).

270   Unlike NH_4^+, and in contrast to our hypotheses, reducing precipitation had no effect on

271average extractable NO_3^- concentrations (Fig. 3; LnR = 0.088 ± 0.16 95% CI) and NO_3^- was not

272related to manipulation length or % precipitation reduction (Fig. S4; *P* > 0.2). Accounting for

273shelter type (Q_M *P* = 0.08) or biome (Q_M *P* = 0.4) in categorical models did not explain a

274significant proportion of the heterogeneity among studies (Table 1).

275*3.4. N_2O emissions*

276   Opposite to our predictions, reducing precipitation significantly lowered N_2O emissions

277across studies (Fig. 3; Hedge's *d* = -0.76 ± 0.62 95% CI). N_2O was not related to manipulation

278length or % precipitation reduction (Fig. S5; *P* > 0.1). Based on categorical models, biome did

279not explain much of the heterogeneity among studies (Table 1; Q_M *P* = 0.7).

280**5. Discussion**

281   We used meta-analysis to determine how experimental reductions in precipitation may

282alter soil N cycling. Because microbes are sensitive to changes in moisture [*Blankinship et al.,*

2832011; *Schimel et al.,* 2007; *Skopp et al.,* 1990] and their activity decreases as soils dry [*Fierer*

284*and Schimel,* 2002; *Manzoni et al.,* 2012; *Orchard and Cook,* 1983], reducing precipitation could

285lower rates of N cycling (Fig. 1A). However, we found that on average, only N_2O emissions

286were lower under precipitation reduction in the field (Figs. 1B and 3) and that the effect of

287reducing precipitation did not vary by biome for the variables of interest, except for tropical sites

288where microbial biomass decreased (Table 1). Reducing precipitation had no effect on N supply

289or NO_3^- pools, and NH_4^+ pools increased on average. Microbial biomass pools demonstrated a

290more dynamic relationship with rainfall manipulation—increasing at lower levels of proportional

291precipitation reduction while decreasing as water was withheld—that was not exclusive to

292Mediterranean regions (Table 1). Together, our results suggest that N supply is relatively less

293sensitive to reduced precipitation, whereas other N cycling processes affecting the balance of

294fluxes and magnitude of N stocks are more sensitive.

295*5.1. N supply*

296   Consistent with our initial hypothesis, reducing precipitation did not have a significant

297effect on N supply, suggesting that microbial processes regulating N supply may be drought

298tolerant relative to other N cycling processes (Fig. 1). Sustained or higher rates of N

299mineralization have been measured in Mediterranean regions where the dry season can last

300several months [*Homyak et al.*, 2016; *Jackson et al.*, 1988; *Parker and Schimel*, 2011], and

301where microbial biomass N increases as soils dry [*Homyak et al.*, 2014; *Parker and Schimel*,

3022011; *Schaeffer et al.*, 2017; *Vourlitis et al.*, 2009], implying microbes maintain some level of N

303processing. N mineralization is carried out by a relatively "broad" range of organisms [*Schimel*

304*and Schaeffer*, 2012], so even if drought caused shifts in microbial community composition, N

305supply could be sustained.

306   We emphasize that small sample sizes prevent a conclusive test of the N supply response

307to drought. Net, gross, anaerobic, and potential N mineralization all measure different aspects of

308N supply, and there were insufficient studies to analyze these metrics individually. By combining

309these different metrics, we gain insight into the qualitative response of N supply to drought, but

310we cannot resolve the underlying mechanisms. For gross, anaerobic, and potential N

311mineralization, a lack of response to precipitation reduction suggests that the process is

312insensitive to drought. However, for *net* N mineralization, insensitivity to drought could also

313result from offsets in gross N mineralization and immobilization (e.g., a decrease in gross N

314mineralization coupled to an increase in N immobilization). Therefore, we cannot rule out the

315possibility that gross N mineralization was sensitive to drought in studies that only measured net

316N mineralization. Nevertheless, the lack of a response to precipitation reduction suggests that,

317relative to N immobilization, gross N mineralization may be less sensitive to drought—NH_4^+

318increased (Fig. 4) while microbial biomass decreased (Fig. 2) as precipitation was withheld. We

319also note there was significant publication bias in studies measuring N supply (Table S2),

320suggesting a negative response to precipitation reduction was more likely to be published than a

321positive response. That we still did not detect a significant effect of reduced precipitation on N

322supply may suggest that our conclusion—that N supply is relatively tolerant of drought stress—

323could be conservative.

324*5.2. Soil microbial biomass*

325   While it is clear that some microbial activities decline as soils dry [*Allison and Treseder*,

3262008; *Manzoni et al.*, 2012; *Ren et al.*, 2017], microbes can tolerate drought stress [*Canarini et*

327*al.*, 2016; *Lennon et al.*, 2012; *Schimel et al.*, 2007], and even increase in biomass [Fig. 2A; *Boot*

328*et al.*, 2013; *Homyak et al.*, 2014; *Jackson et al.*, 1988; *Parker and Schimel*, 2011; *Ren et al.*,

3292017; *Schaeffer et al.*, 2017]. Indeed, a recent meta-analysis suggests that microbial biomass is

330less likely to decrease in response to reducing precipitation in dry (MAP ≤600 mm) relative to

331wet ecosystems [*Ren et al.*, 2017]. Across the studies included in our analysis that reduced

332precipitation by relatively small proportions, a shift in the frequency of precipitation pulses could

333have increased microbial growth due to greater numbers of wet-dry cycles [*Fierer and Schimel*,

3342002; *Xiang et al.*, 2008]. Such a response could occur if deploying rain-out shelters for

335relatively short periods alters the frequency of precipitation pulses [*Huxman et al.*, 2004] while

336having relatively little effect on total precipitation. In addition, microbes might survive in

337hydrologically disconnected microsites formed as soils dry despite bulk soils having negligible

338diffusivity at the macro scale [*Manzoni and Katul*, 2014]. Microbes surviving in these microsites

339may gain protection from predators and/or viruses, and reduced *mortality* rather than increased

340*activity* may explain the increased biomass [*Parker and Schimel*, 2011; *Ranjard and Richaume*,

3412001]. Changes in community structure or dormancy may also explain shifts in the size of the

342microbial biomass pool in drying soils [*Fuchslueger et al.*, 2014a; *Fuchslueger et al.*, 2016;

343*Lennon and Jones*, 2011]. Sullivan et al. [2012] noted that archaea may fare better than bacteria

344at low water potential [but see *Fuchslueger et al.*, 2014b], whereas other studies suggest drought

345tolerant fungi may continue to produce biomass [*Treseder et al.,* 2010; *Yuste et al.,* 2011]. One or

346more of these dynamics may explain why the size of the microbial biomass pool increased with

347small reductions in precipitation and, therefore, why microbial processes continued to mineralize

348N.

349   In contrast to the increase in microbial biomass when precipitation was reduced by small

350fractions, microbial biomass decreased as precipitation reduction approached 100 % (Fig. 2A).

351Reducing precipitation by large percentages (i.e., deploying rain-out shelters for extended

352periods) reduced average soil moisture as well as the frequency of precipitation pulses. These

353persistently dry conditions could have desiccated soils beyond thresholds of microbial drought

354tolerance—about -14 MPa for mineral soils and -36 MPa for litter [*Manzoni et al.,* 2012]. This

355relationship was partly driven by responses to drought in the tropics, where microbial biomass

356declined particularly strongly at 100% precipitation reduction (Table 1). If we exclude tropical

357studies from the continuous meta-analysis of microbial biomass versus % precipitation reduction,

358a significant relationship is no longer observed ($P = 0.5$ without tropical studies). Although more

359studies would be required to draw firm conclusions about cross-biome variation, microbes

360adapted to moist tropical environments could be more sensitive to drought stress than microbes

361from more variable environments [*Hawkes and Keitt,* 2015].

362*5.3. Extractable NH_4^+*

363   We observed that extractable NH_4^+ increased with greater precipitation reduction (Fig.

3644A). Because microbial biomass decreased as precipitation reduction approached 100 %,

365microbial death might have contributed to the increase in NH_4^+ and sustained N supply. However,

366since N supply did not significantly increase, other mechanisms probably contributed to the

367increase in NH_4^+. In particular, low water availability can limit plant growth [*Shi et al.,* 2014; *Wu*

368 *et al.,* 2011], plant NH_4^+ uptake [*Zhong et al.,* 2014], and nitrification rates [*Stark and Firestone,*

369 1995; *Wetselaar,* 1968]. The balance of minor changes in these potential mechanisms, along with

370 our observations, suggest that extractable NH_4^+ increases with drought treatment due to reduced

371 NH_4^+ consumption (Fig. 1B).

372 *5.4. Extractable NO_3^-*

373   The pool of extractable NO_3^- did not change with reduced precipitation (Fig. 3), even

374 though other drivers of NO_3^- concentrations were likely affected (e.g., NH_4^+ concentrations,

375 nitrification, plant N uptake, and denitrification). Nitrification declines under low osmotic

376 potentials [*Stark and Firestone,* 1995], and in drying soils a larger fraction of nitrified N can

377 escape as NO [Fig. 1B; *Davidson et al.,* 2008; *Homyak et al.,* 2016; *Homyak et al.,* 2017], which

378 together imply NO_3^- production could have declined. Similarly, the processes consuming NO_3^-

379 probably also declined; plant NO_3^- uptake declines under drought stress [*Dijkstra et al.,* 2015;

380 *Meng et al.,* 2016] as do N_2O emissions (Fig. 3). Thus, NO_3^- concentrations may not have

381 changed because reductions in production and consumption of NO_3^- offset one another (Fig. 1B).

382 *5.5. N_2O emissions*

383   Reducing precipitation significantly lowered N_2O emissions across studies (Fig. 3),

384 perhaps suggesting that denitrification is more sensitive to drought than processes controlling N

385 supply. Because shifts in the ratio of oxidant (e.g., NO_3^-) to reductant (organic C) can influence

386 whether N_2O or N_2 is produced during denitrification [*Del Grosso et al.,* 2000], lower N_2O fluxes

387 may reflect shifts in the ratio of $N_2O:N_2$ produced, not lower denitrification rates. The effect of

388 reduced precipitation on denitrification is likely contingent upon ecosystem-specific factors that

389 can both lower and even increase N_2O emissions as precipitation is withheld [*Wieder et al.,*

390 2011]. For instance, potential denitrification enzyme activity [*Parker and Schimel,* 2011] and

391both NO_3^- and water-extractable organic C concentrations [*Schaeffer et al.*, 2017] can increase as

392soils dry, presumably influencing N_2O emission rates during drying and/or upon rewetting soils.

393Nevertheless, our finding is consistent with greater denitrification under wet, anaerobic

394environments [*Robertson and Groffman*, 2007], because these conditions would have been less

395common under precipitation reduction. Compared to microbes that mineralize N, denitrifiers are

396more narrowly distributed phylogenetically [*Schimel and Schaeffer*, 2012] and require wetter

397conditions to maintain metabolic activity [e.g., *Lennon et al.*, 2012]. Our results are consistent

398with studies reporting low N_2O emissions in drylands except during short periods following

399precipitation pulses when soils wet up [*Soper et al.*, 2016a; *Soper et al.*, 2016b] or when fast

400chemical reactions may occur [*Heil et al.*, 2015].

401*5.6. Implications for N cycling under drought*

402   Our results expand on previous laboratory studies evaluating N dynamics in dry soils.

403While it is clear that microbial N transformations decrease at low water potentials [*Campbell*

404*and Biederbeck*, 1972; *Pilbeam et al.*, 1993; *Reichman et al.*, 1966; *Stark and Firestone*, 1995],

405we show that under *in-situ* field conditions, processes that control N supply are less sensitive to

406precipitation reduction. Differences between field and laboratory studies likely stem from the

407artificial effects on water infiltration and oxygen diffusion generated by disrupting soil

408aggregates in microcosms, as well as by microbial interactions with soil physical and biological

409factors not well represented in the laboratory. For instance, interactions between plants and

410microbes shape soil aggregates [*Blankinship et al.*, 2016], where microbes can remain hydrated

411in microsites despite low bulk soil water potential [*Manzoni and Katul*, 2014; *Parker and*

412*Schimel*, 2011]. Moreover, non-rainfall water inputs, such as the movement of atmospheric water

413vapor into soil, can stimulate drought-tolerant microbial processes in the field [*McHugh et al.*,

4142015], and may maintain active microbial N processing despite low soil water content. Together,

415these microbial interactions with physical and biological factors, along with the relative tolerance

416of N mineralization to drought stress [*Booth et al.*, 2005; *Fisher et al.*, 1987; *Pilbeam et al.*,

4171993; *Robinson*, 1957], are consistent with the findings of our meta-analysis and may explain

418discrepancies between field and laboratory studies.

419   Our meta-analysis is constrained by the number of precipitation reduction studies

420available to assess effects on soil N cycling, particularly for N supply. Assuming both a small

421sample size and high variation between treatment and control plots within studies, using the last

422observation in a time-series to calculate an effect size—a standard meta-analytical approach—

423may bias interpretations. For instance, *Davidson et al.* [2008] reported lower N_2O emissions in

424rainfall exclusion plots than in control plots at the end of the experimental manipulation, but had

425the experiment ended about two years earlier, we would have calculated the opposite effect size,

426albeit small. Because we synthesized field studies, we expected high variation between and

427within sites, especially since soils under precipitation reduction treatment could have moisture

428levels similar to control soils if the % reduction in precipitation was small. To assess how

429manipulation length may have influenced our conclusions, we show with continuous meta-

430analyses that, across all studies and variables of interest, manipulation length did not

431significantly influence effect size ($P \geq 0.2$).

432   At the ecosystem scale, our analyses help inform predictions about N cycling in regions

433where drought magnitude and duration may increase [e.g., *Delgado-Baquerizo et al.*, 2013].

434Drought magnitude and duration should influence whether ecosystems operate as net N sinks or

435sources. A relatively mild drought would be expected to produce a small pool of bioavailable N

436[Fig. 4A; *Hartmann et al.*, 2013], since ecosystem N sinks (e.g., microbial and plant N uptake

437and soil organic matter) may persist or recover quickly from drought [e.g., *de Vries et al.*, 2012;

438*Fry et al.*, 2014; *Homyak et al.*, 2014; *Mauritz et al.*, 2014]. As drought severity increases,

439however, a larger mineral N pool, together with drought-stressed ecosystem N sinks may

440generate responses similar to those following ecosystem disturbance, favoring larger N losses via

441N gas evasion and leaching upon rewetting [e.g., *Evans and Burke*, 2013; *Hanan et al.*, 2016;

442*Homyak et al.*, 2016; *Homyak et al.*, 2017; *Homyak et al.*, 2014]. The temporal sequence of these

443different magnitudes of dry conditions may control the long-term changes in ecosystem N

444cycling transformations, fluxes, and stocks.

445**6. Conclusions**

446   We did not detect significant changes in the rate of N supply in response to precipitation

447reduction, apparently as a result of microbes enduring through dry conditions. Because of the

448relatively low number of drought manipulation experiments, it is critical for ongoing and future

449studies to include N transformation measurements, as these data are urgently needed for

450calculating thresholds of microbial drought tolerance and N processing. We also emphasize

451reporting soil bulk density to facilitate calculation of water-filled pore space and allow

452comparisons of soil water content across sites varying in soil texture. Our analyses imply

453differences in drought sensitivity among N cycling processes, with processes controlling the rate

454of N supply appearing less sensitive to drought conditions than processes controlling N_2O

455emissions. Microbial biomass declines and NH_4^+ accumulates as drought intensity increases,

456consistent with reduced plant and microbial uptake from mineral N pools. One implication of

457this finding is that increasing drought magnitude and duration, through sustained N supply, could

458control ecosystem N losses during subsequent wet-up periods.

459 **Acknowledgments**

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682**Tables**

683Table 1. Results of statistical comparisons among and within groups (MB = microbial biomass; [†]only groups with two or more studies

684were included in comparisons)

Parameter	Group	Sub-group	LnR	95% CI	n	Q_M	Q_E	*P*-value among groups[†]
MB	All studies		-0.042	-0.22 to 0.13	27			
	Shelter type					0.003	26.53	0.953
		Exclusion	-0.044	-0.24 to 0.16	22			
		Reduction	-0.030	-0.61 to 0.55	5			
	Extraction					16.41	19.56	0.0003
		PLFA	0.49	-0.15 to 1.13	4			
		$CHCl_3$	-0.042	-0.23 to 0.15	18			
		SIR	-0.62	-1.22 to -0.019	4			
	Biome					8.85	18.23	0.065
		Agriculture	-	-	-			
		Forest	0.16	-0.19 to 0.50	9			
		Grassland	0.18	-3.37 to 3.73	2			
		Meadow	0.099	-3.52 to 3.71	2			
		Shrubland	0.024	-0.60 to 0.65	5			
		Tropical	-0.41	-0.77 to -0.058	8			
	Climate					0.10	26.40	0.750
		Mediterranean	-0.10	-0.68 to 0.47	5			
		Non-Mediterranean	-0.029	-0.23 to 0.17	22			
NH_4^+	All studies		0.22	0.0033 to 0.43	24			
	Shelter type					0.12	25.84	0.733

Parameter	Group	Sub-group	Hedge's d	95% CI	n	QM	QE	*P*-value among groups[†]
		Exclusion	0.19	-0.068 to 0.45	18			
		Reduction	0.27	-0.23 to 0.78	6			
	Biome					2.04	18.33	0.564
		Agriculture	-	-	-			
		Forest	0.52	-0.77 to 1.80	3			
		Grassland	0.40	-0.32 to 1.11	5			
		Meadow	0.22	-1.06 to 1.51	3			
		Shrubland	0.073	-0.34 to 0.49	11			
		Tropical	-	-	-			
NO_3^-	All studies		0.088	-0.071 to 0.25	24			
	Shelter type					3.07	29.04	0.08
		Exclusion	0.012	-0.17 to 0.20	18			
		Reduction	0.32	-0.074 to 0.72	6			
	Biome					2.92	14.13	0.404
		Agriculture	-	-	-			
		Forest	0.061	-1.37 to 1.49	3			
		Grassland	0.066	-0.65 to 0.78	5			
		Meadow	-0.28	-1.59 to 1.03	3			
		Shrubland	0.35	-0.13 to 0.83	11			
		Tropical	-	-	-			

Parameter	Group	Sub-group	Hedge's d	95% CI	n	QM	QE	*P*-value among groups[†]
N supply	All studies		0.028	-0.43 to 0.48	16			
	Shelter type					0.66	19.99	0.415
		Exclusion	-0.10	-0.70 to 0.49	11			
		Reduction	0.27	-0.76 to 1.30	5			
	Biome					6.73	13.82	0.035
		Agriculture	-	-	-			
		Forest	-	-	-			
		Grassland	0.16	-0.92 to 1.24	5			

		Meadow	-1.52	-4.16 to 1.11	3		
		Shrubland	0.21	-0.61 to 1.03	6		
		Tropical	-	-	-		
N_2O	All studies		-0.76	-1.37 to -0.14	15		
	Shelter type					-	-	-
		Exclusion	-	-	-		
		Reduction	-	-	-		
	Biome					0.69	10.34	0.707
		Agriculture	-	-	-		
		Forest	-	-	-		
		Grassland	-1.25	-4.26 to 1.76	3		
		Meadow	-	-	-		
		Shrubland	-0.60	-2.02 to 0.82	5		
		Tropical	-1.069	-2.76 to 0.62	4		

686**Figure legends**

687Fig. 1. Conceptual model for N cycling in dry soils. **A)** The hypothesized effects of dry

688conditions on soil N cycling, versus **B)** the results of our meta-analyses. Pools of soil organic

689matter (SOM), microbial biomass (MB), ammonium (NH_4^+), and nitrate (NO_3^-) are represented

690by boxes. Fluxes are represented by arrows including N supply, nitric oxide (NO), and nitrous

691oxide (N_2O). Dotted arrows represent fluxes most sensitive to diffusion constraints as drought

692severity increases. An increase in the size of the pool or flux is represented by +, a decrease by -,

693and no change by =. [#]Plant growth and N uptake decrease in response to reducing precipitation

694(Zhong et al., 2014; Shi et al., 2014; Wu et al., 2011) whereas NO emissions increase because of

695tradeoffs between biotic and abiotic processes (Homyak et al., 2016).

696Fig. 2. Natural logarithm of the response ratio (LnR) of soil microbial biomass as a function of

697precipitation reduction (% of mean annual precipitation; LnR = -0.009 × (% precipitation

698reduction) + 0.4021; P = 0.0002; intercept P = 0.005). Symbols represent LnR of individual

699studies ± the variance of LnR. The gray square is the grand mean effect size across all studies ±

70095% confidence interval.

701Fig. 3. Mean effect size ± 95% confidence intervals for the effects of precipitation reduction on

702N supply (Hedge's d), N_2O emissions (Hedge's d), and extractable NO_3^- (LnR). LnR = natural

703logarithm of the response ratio.

704Fig. 4. Natural logarithm of the response ratio (LnR) of soil extractable NH_4^+ as a function of

705precipitation reduction (% of mean annual precipitation; LnR = 0.0142 × (% precipitation

706reduction) - 0.4208; P = 0.0003; intercept P = 0.03). Symbols represent the LnR of individual

707studies ± the variance of the LnR. The gray square is the grand mean effect size across all studies
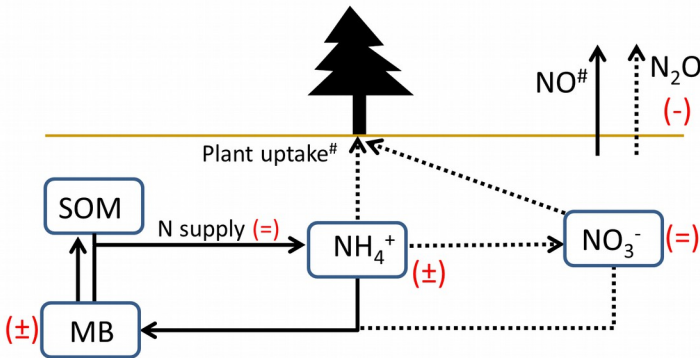
708± 95% confidence interval.

709**Figures**

710*Fig. 1*

A) Hypothesized effects
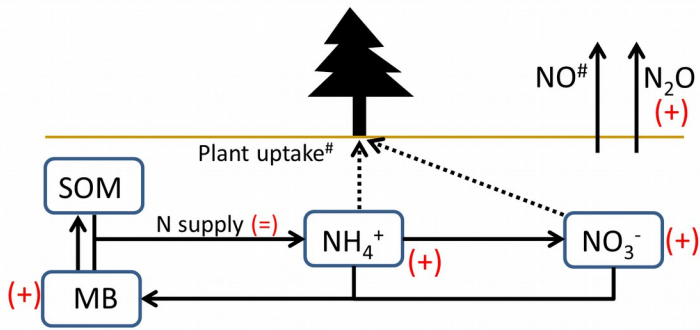
B) Observed effects

711

712Fig. 1. Conceptual model for N cycling in dry soils. **A)** The hypothesized effects of dry

713conditions on soil N cycling, versus **B)** the results of our meta-analyses. Pools of soil organic

714matter (SOM), microbial biomass (MB), ammonium (NH_4^+), and nitrate (NO_3^-) are represented

715by boxes. Fluxes are represented by arrows including N supply, nitric oxide (NO), and nitrous

716oxide (N_2O). Dotted arrows represent fluxes most sensitive to diffusion constraints as drought

717severity increases. An increase in the size of the pool or flux is represented by +, a decrease by -,

718and no change by =. [#]Plant growth and N uptake decrease in response to reducing precipitation

719(Zhong et al., 2014; Shi et al., 2014; Wu et al., 2011) whereas NO emissions increase because of

720tradeoffs between biotic and abiotic processes (Homyak et al., 2016).

721*Fig. 2*
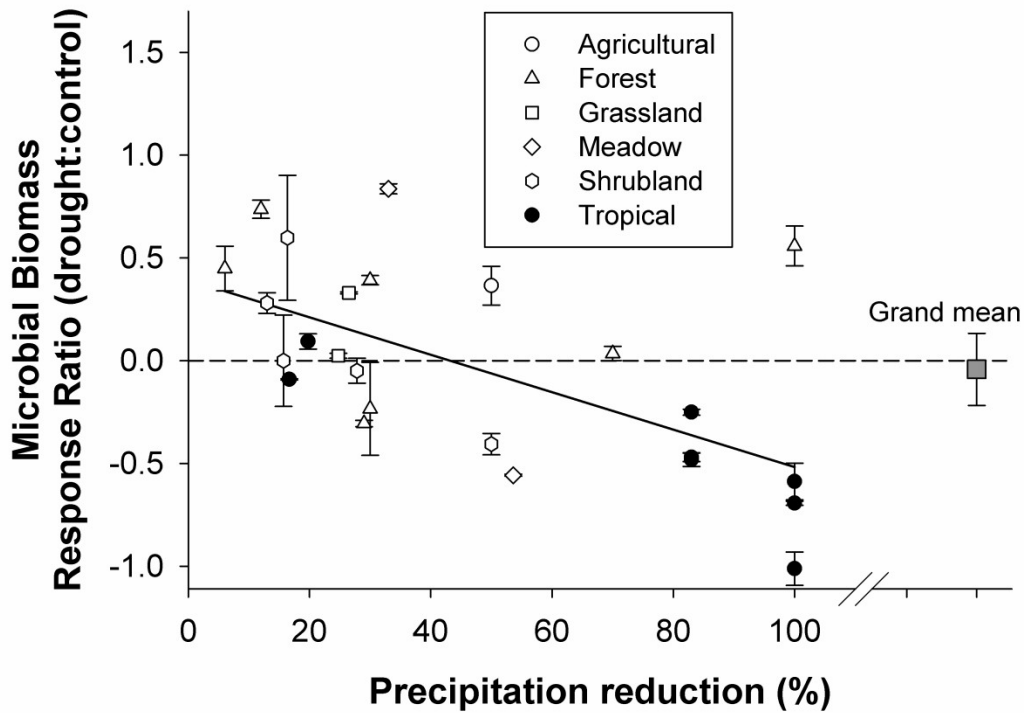
722

723Fig. 2. Natural logarithm of the response ratio (LnR) of soil microbial biomass as a function of

724precipitation reduction (% of mean annual precipitation; LnR = -0.009 × (% precipitation

725reduction) + 0.4021; *P* = 0.0002; intercept *P* = 0.005). Symbols represent LnR of individual

726studies ± the variance of LnR. The gray square is the grand mean effect size across all studies ±

72795% confidence interval.

728*Fig. 3*
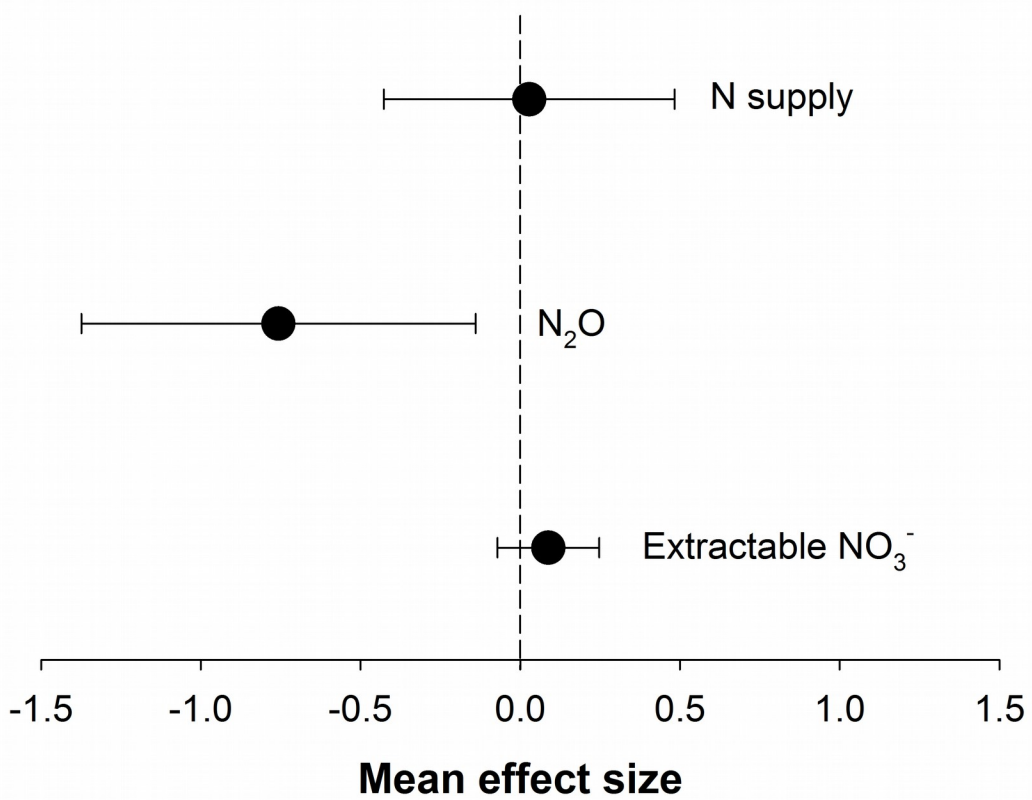
729

730Fig. 3. Mean effect size ± 95% confidence intervals for the effects of precipitation reduction on

731N supply (Hedge's *d*), N_2O emissions (Hedge's *d*), and extractable NO_3^- (LnR). LnR = natural

732logarithm of the response ratio. N supply represents the combined response of net, gross,

733anaerobic, and potential N mineralization to reduced precipitation (see section 2.2).

734*Fig. 4*
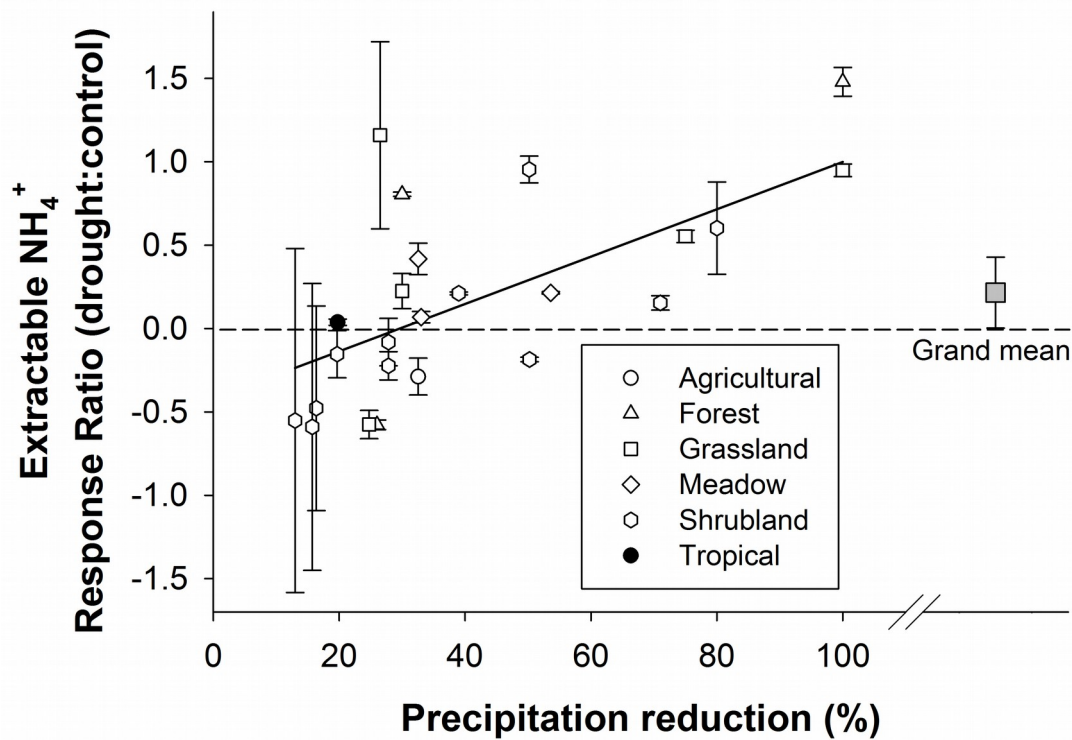
735

736Fig. 4. Natural logarithm of the response ratio (LnR) of soil extractable NH_4^+ as a function of

737precipitation reduction (% of mean annual precipitation; LnR = 0.0142 × (% precipitation

738reduction) - 0.4208; P = 0.0003; intercept P = 0.03). Symbols represent the LnR of individual

739studies ± the variance of the LnR. The gray square is the grand mean effect size across all studies

740± 95% confidence interval.