Response to nitrogen addition reveals metabolic and ecological strategies of soil bacteria

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Summary

The nitrogen (N) cycle represents one of the most well studied systems yet the taxonomic diversity of the organisms that contribute to it is mostly unknown, or linked to poorly characterized microbial groups. While progress has allowed functional groups to be refined, they still rely on *a priori* knowledge of enzymes involved, and the assumption of functional conservation, with little connection to the role the transformation plays for specific organisms. Here, we use soil microcosms to test the impact of N deposition on prokaryotic communities. By combining chemical, genomic and transcriptomic analysis we are able to identify and link changes in community structure to specific organisms catalyzing given chemical reactions. Urea deposition led to a decrease in prokaryotic richness, and a shift in community composition. This was driven by replacement of stable native populations, which utilize energy from N-linked redox reactions for maintenance, with fast responding populations that use this energy for growth. This model can be used to predict response to N disturbances and allows us to identify putative life strategies of different functional, and taxonomic, groups thus providing insights into how they persist in ecosystems by niche differentiation.

40

41 **Introduction**

42 Modern microbiological techniques have given us unprecedented access to the
43 microbial world (Spiro, 2012; Rinke *et al.*, 2013), yet soil communities remain poorly
44 understood (Delmont *et al.*, 2015). While many studies have focused on the diversity
45 or abundance of key populations (Taylor *et al.*, 2012; Gubry-Rangin *et al.*, 2015; Wei
46 *et al.*, 2015), fewer have looked at the transcriptional profiles over time (Nicol *et al.*,
47 2008; Morales and Holben, 2013), and even less have done so for multiple groups at
48 the same time (Liu *et al.*, 2010; 2014; Brenzinger *et al.*, 2015). This is particularly
49 true of organisms involved in nitrogen (N) cycling in soils. The complexity of the
50 underlying processes combined with the diversity of microbes contributing to each
51 process provides a large challenge to identifying mechanisms active at any given time
52 (Butterbach-Bahl *et al.*, 2013). Currently we lack enough information to understand
53 basic ecological concepts linked to N cycling *in situ* such as: i) substrate competition
54 at both inter and intra species level, ii) full diversity of both present and active N
55 cycling populations, iii) and the life strategies of these populations which in turn
56 control their responses (both as observed growth or transcriptional changes).

57 Where progress has been made, groundbreaking findings have followed. The
58 initial discovery of ammonia oxidizing archaea (AOA) and its subsequent rise to
59 major player in the N cycle (Leininger *et al.*, 2006; Hatzenpichler, 2012; Stahl and la
60 Torre, 2012) highlighted the unexpected gaps in knowledge. Later studies have
61 suggested different life strategies for AOA when compared to ammonia oxidizing
62 bacteria (AOB) (Sterngren *et al.*, 2015), but this may be complicated by variance
63 across strains (Bayer *et al.*, 2015). One major unknown is whether observations made
64 in studies, or organisms, from one ecosystem translate to others.

65 It is well established that individual intermediates in the N cycle can be used
66 for specific reasons (i.e. ammonia oxidation provides electrons, while denitrification
67 intermediates accept reducing equivalents), but the purpose of the reactions for any
68 organism is another major unknown. That is, while some organisms carry out these
69 processes for electrogenic purposes that can result in growth, others do it in order to
70 maintain redox homeostasis (Simon and Klotz, 2013). Unfortunately examples where
71 an organism harbours multiple versions of the same enzyme for completely different
72 purposes (respiration vs. redox balance) exist (Hartsock and Shapleigh, 2011), and are
73 likely to limit generalizations.

74 Despite this, studies focusing on population changes in response to
75 manipulations have consistently recorded conserved patterns (e.g. growth of AOB but
76 not AOA (Jia and Conrad, 2009; Di *et al.*, 2009; Pratscher *et al.*, 2011)) suggesting
77 that responses by specific populations in a given location or ecosystem are
78 predictable. However, the debate continues on whether niche specialization and
79 differentiation can be determined based solely on correlations, without analyzing the
80 wider array of processes that contribute or influence any given N transformation
81 (Prosser and Nicol, 2012). This is relevant in ecosystems such as agricultural
82 grassland where an understanding of N cycling is crucial for management of both
83 productivity and greenhouse gases (Herrero *et al.*, 2016), of which nitrous oxide
84 (N_2O) is a key player (Reay *et al.*, 2012).

85 In grazed pastures (i.e. agricultural grasslands) N deposition through ruminant
86 urine drives the emissions of N_2O (Saggar *et al.*, 2013). In this system a full cascade
87 of transformations begin with urea and can result in accumulation of any intermediate
88 depending on conditions, but with a final end product of N_2 or N_2O. While the
89 chemical transformations have been explored (Hamonts *et al.*, 2013; Baral *et al.*,

2014; de Klein, Shepherd, *et al.*, 2014; de Klein, Luo, *et al.*, 2014), mechanistic understanding of the populations catalyzing the reactions, and the purpose they serve for the organisms is less clear. In this study we aimed to identify active biochemical pathways involved in N loss (through gases) in response to urea (simulated ruminant urine deposition event) and varying moisture content. Observed phenotypes (chemical transformations) were linked to changes in genotype (functional potential through DNA; a proxy for population changes), expression of genotype (RNA profiles), and total community composition (specific taxonomically defined populations based on 16S). We hypothesized that sequential transformation of nitrogenous intermediates would be coupled to changes in expression of functional genes catalyzing production and consumption of intermediates. Alternatively, transformations not linked to population, or expression changes, would be driven by other (non-biochemical) pathways. We also hypothesized that changes in transcription, or population size, could serve to determine life strategies of microbes utilizing each intermediate (whether they are used for growth vs. maintenance). To test this we mimicked a ruminant urine-N deposition event using repacked soil cores (1.1 mg m^{-3}) on tension tables monitored for 63 days. Soils were treated with urea under two different moisture contents: high (near saturation; -1.0 kPa) and low (field capacity; -10 kPa) moisture. Simultaneous measurements of soil chemistry, gas kinetics, microbial community composition (by 16S amplicon sequencing) and functional gene abundance (for nitrification and denitrification) at DNA (gene) and RNA (transcript) levels were performed to determine the active populations and pathways.

1.

Results:

Soil pH and N transformation dynamics in response to urea

Soil pH increased from acidic (pH = 5.5) to alkaline reaching a maximum (pH = 8.5) at day 3 in urea treated soils. Return to baseline pH was modulated by soil moisture with high moisture (HM; -1.0kPa) soil reaching baseline at day 35 and low moisture soils (LM; -10kPa) doing so at day 53 (Fig. 1). This shift in pH was linked to a successive N transformation process initiated with urea hydrolysis and leading to nitrification and denitrification: urea → NH_4^+ → NO_2^- → NO_3^- → N_2O → N_2 (Fig. 1). Sequential peak activity was observed for each transformation with the response modified by moisture. Maximum production (mean μg N g^{-1} soil) for each transformation was observed at day 3, 21 and 35 respectively for NH_4^+ (HM+N = 1758; LM+N= 1730), NO_2^- (HM+N = 79.2; LM+N= 39.7) and NO_3^- (HM+N = 429.2; LM+N= 335). Two distinct production peaks were observed for N_2O, with a short pulse (0 to 5 days) reaching a maximum at day 2 for HM soils (11602.8 μg m^{-2} h^{-1}) and day 3 for LM soils (46.8 μg m^{-2} h^{-1}) (Fig. 1 and Supplementary Fig. S1). A second, longer duration (10 to ~50 days), N_2O pulse reached a maximum at day 28 for HM soils (6405.1 μg m^{-2} h^{-1}) and day 30 for LM soils (448.9 μg m^{-2} h^{-1}). The large N_2O spike (first peak) between days 0 to 5 in the HM+N treatment was about 11.6% of the total N_2O cumulative flux over 63 days, whereas in the LM+N treatment the 0 to 5 day periods accounted for 22.3% of the total N_2O cumulative flux over 63 days.

Population and transcription dynamics for nitrogen related functional groups

136 Significant changes (ANOVA, $p<0.05$) in relative activity (mRNA copy
137 numbers/16S rRNA gene copy numbers) were observed promptly between day 0 & 3
138 for all functional groups (except AOA and N-fixers in HM soil) in response to urea
139 (Fig. 1). However, maximum relative transcription did not match maximum
140 production peaks for corresponding substrates, or products, for each functional group.
141 Nitrifiers (ammonia oxidizers) displayed niche differentiation, with time, length and
142 strength of response differing between bacterial (AOB) and archaeal ammonia
143 oxidizers (AOA). Relative activity of AOA increased (4.6-fold for LM and 1.6-fold
144 for HM) under urea treatments at day 3 only, with a subsequent decrease (-19.3-fold
145 for LM and -7-fold for HM) resulting in lower expression than in untreated soils (Fig.
146 1). AOB relative activity also increased but was sustained for a much longer period
147 (3-63 days), with maximum activity (>11-fold change) seen at 21 and 35 days for
148 HM+N and LM+N respectively (Fig. 1). Denitrifiers (both nitrite and nitrous oxide
149 reducers) showed similar responses as AOA, with peak activity at day 3 and a rapid
150 return to baseline, in the case of nitrite reducers decreasing to levels below those
151 observed in non-urea treated soils (Fig. 1). To account for endogenous sources of N,
152 N_2 fixers were monitored through the activity of the nitrogenase gene (*nifH*). No
153 significant changes were observed except for day 3 (LM +N only), with a subsequent
154 decrease in activity below background. This decrease below background was
155 observed for all N treated samples.

156 Changes in the relative contribution to total community composition were
157 calculated by normalizing functional gene copy numbers to total 16S rRNA gene copy
158 numbers per sample for each functional group (Fig. 1). The maximum observed
159 relative abundance of each functional group differed for each group (HM and LM
160 respectively): AOB, 19 & 12 %; AOA, 8 & 13 %; *nirS*, 6.3 & 2.9 %; *nosZI*, 3.3 & 3.4

161 %; *nifH*, 4.7 & 4.32 %. Further, large population changes over time were mostly
162 limited to AOB. Generally, AOB comprised <1 % of the total community, but in
163 response to urea increased up to 29-fold to make up 19 % (day 21 for HM) and 20-
164 fold to make up 12 % (day 35 for LM) of the community in urea treated soils. In
165 contrast, AOA were found at consistently high levels (median=4.2 %) in untreated
166 soils, but numbers decreased >7-fold in response to urea (~1.3 % at least 63 day).
167 Similarly, other functional groups (*nosZI*, *nifH*) decreased or remained stable (*nirS*) in
168 response to urea. Similar patterns for both activity and population changes were
169 observed when absolute values were analyzed (Supplementary Fig. S2).

170

171 *N deposition induces both a genotypic and a transcriptional response at the*
172 *community level that is modified by soil moisture content*

173 Urea deposition imposed a general negative selective pressure leading to
174 decreases in OTU level prokaryotic diversity (Shannon, -1.2-fold change) and
175 richness (-1.5-fold change) at DNA level (Fig. 2a, Supplementary Fig. S3). The same
176 pattern was observed when active microbes (based on RNA) were analyzed with
177 decreases in OTU level prokaryotic diversity (Shannon, -1.3-fold change) and
178 richness (-1.9-fold change). Moisture was found to play a modifier role, with LM
179 samples consistently resulting in lower diversity and richness when compared to their
180 HM pairs. Richness and diversity losses were not recovered even after 63 days. In
181 contrast, samples where no urea was applied remained stable (i.e. constant diversity
182 and richness).

183 Urea deposition significantly altered community structure as shown in a non-
184 metric multidimensional scaling (NMDS) plot using a Bray-Curtis dissimilarity

185 matrix (Fig. 2b and Supplementary Fig. S4). At both DNA and RNA level community
186 changes along the first axis corresponded with changes in response to urea treatment,
187 with the second axis accounting for changes in moisture. A pvclust analysis
188 (hierarchical clustering with p-values calculated via multiscale bootstrap resampling,
189 Supplementary Fig. S5) confirmed two major clusters [100% AU (Approximately
190 Unbiased) and 100% BP (Bootstrap Probability)] formed by urea treated (HM+N and
191 LM+N samples, excluding day 0), vs. untreated soils (HM-N, LM-N, field samples,
192 and HM+N & LM+N at Day 0). Temporal variance within each cluster was
193 confirmed using a Mantel correlogram analysis (Fig. 2c). Urea treated samples had
194 significant changes in community composition immediately upon treatment (Day 0 to
195 7), with no return to baseline conditions by the end of the experiment. In contrast,
196 untreated samples did not change significantly over time (Supplemental Fig. S6)

197 Changes in community structure were associated with shifts in major
198 taxonomic lineages (Fig. 3). In general, phylum level changes in abundance and
199 transcription where correlated to each other (Supplementary Table S2 and Fig. S7,
200 S8). Urea deposition induced temporal changes in phylum level abundance with
201 observed maximum fold changes per group (HM & LM at DNA level) being:
202 Acidobacteria, -4.6 & -3.7; Actinobacteria, 2.4 & 5.3; Bacteroidetes, 4.6 & 2.2;
203 Candidate Division WS3, -10.5 & -7; Chloroflexi, -2.9 & -2.6; Firmicutes, 10.8 &
204 16.2; Gemmatimonadetes, 2 & 3.3; Nitrospirae, -3.2 & -2; Planctomycetes, -3.7 & -
205 2.5; Thaumarchaeota, -5.2 & -3.6; Verrucomicrobia, -2.5 & -2; Alphaproteobacteria,
206 1.4 & 1.7; Betaproteobacteria, 4 & 2; Deltaproteobacteria, -2.2 & -1.4;
207 Gammaproteobacteria, 1.5 & 2.6. Normalized transcriptional activity (reads of 16S
208 rRNA/reads of 16S rDNA) identified the Firmicutes and members within classes of
209 the Proteobacteria as the most transcriptionally active. While abundant phyla tended

210 to have high levels of normalized transcription, less abundant organisms like the
211 Thaumarchaeota, were observed to have high normalized transcriptional activity
212 especially under background conditions (Supplementary Fig. S7). In contrast, groups
213 traditionally considered slow growers (e.g. Nitrospirae and Gemmatimonadetes) had
214 low normalized transcription. It was also noted that while normalized transcription
215 levels remained stable without urea, N deposition induced changes. These changes in
216 normalized activity did not always match trends observed at individual DNA or RNA
217 level (e.g. Firmicutes).
218
219 *Shifts in N and moisture status trigger OTU response linked to divergent life*
220 *strategies*
221
222 Since Fig. 3 only represents a taxonomic summary of all OTUs (irrespective
223 of their response to treatments), it does not provide a clear indication of who is
224 changing and why. To account for this, urea responsive OTUs were identified
225 independently in RNA and DNA profiles (under each treatment) through a SIMPER
226 analysis. OTUs accounting for 50% of the variance were analyzed (Fig. 4). Response
227 patterns for detected OTUs were conserved between RNA and DNA profiles.
228 However, while some OTUs responded similarly to urea under varying moisture
229 conditions, marked differences were observed with no detectable pattern based on
230 taxonomy.
231 OTUs within the Proteobacteria identified in the SIMPER analysis did not
232 display a conserved response to urea, however when lower taxonomic levels were
233 examined patterns emerged. A consistent positive response was seen for OTUs within
234 the class Betaproteobacteria and the family Hyphomicrobiaceae, amongst others.

235 Conserved positive responses to urea where also observed at the phylum level for the
236 Firmicutes, Bacteroidetes, Actinobacteria, Gemmatimonadetes and Planctomycetes,
237 although the level of response varied across lower taxonomic levels. In contrast, with
238 only some exceptions, OTUs within the phyla Acidobacteria, Verrucomicrobia,
239 Nitrospirae, Candidate Division WS3 (also referred to as candidate phylum
240 Latescibacteria) and the Thaumarchaeota all were negatively impacted by urea
241 deposition.

242 To account for response patterns over time, we focused on OTUs that
243 accounted for 30% of the variance in the SIMPER analysis (36 total), with individual
244 OTU contributions ranging from 5 to 0.1 percent at the DNA level and 5 to 0.06
245 percent at the RNA level (Table S3). Temporal patterns were conserved between
246 DNA and RNA profiles (Supplementary Fig. S9, S10), despite differences in absolute
247 abundance. Once again, moisture acted as a modulator of response with the extent of
248 impact dependent on the OTU (Fig. 5 and 6). While most functional groups responded
249 immediately (at both DNA and RNA level), positively affected OTU responses were
250 observed along all time points creating a succession of positively selected organisms.
251 In contrast, negatively affected OTUs all responded within the first 2 time points
252 indicating an immediate negative selective pressure (Fig. 6). Large variances in
253 absolute changes were observed, even within similar organisms (e.g. Pedobacter),
254 with fold changes ranging from -10.5 to 410 across both positively and negatively
255 affected OTUs. Despite this, OTU response was noted to correspond to taxonomy,
256 with both the effect (positive or negative) and the extent of response (fold change or
257 total abundance) in line with predicted ecological growth strategies (r vs. k) predicted
258 for different taxa. To test this, we predicted rRNA operon copy numbers (rrn) for all
259 36 OTUs and compared them to the observed maximum abundance, max fold change

260 in population or observed growth rate per day. We consistently observed a non-linear
261 response with an asymptote reached at higher copy numbers (Fig. 7). These trends
262 were consistent independent of which moisture conditions were present at the time of
263 response. To account for preferential response due to moisture, we selected the
264 highest response for each organism and saw no clear difference in patterns. To
265 account for potential biases due to uneven representation, OTUs were grouped into
266 low (1-2 copies of rrn) or high (>2) copy number organisms (Supplementary Table
267 S4). While significant changes ($p < 0.05$, Supplementary Fig. S11) were observed in
268 most instances, exceptions were noted (e.g. growth rate under HM).
269
270 **Discussion**
271 Functional profiling (identification and quantification of specific functional
272 genes/transcripts) is normally utilized to link chemical transformations to specific
273 microbial populations capable of catalyzing reactions. However, functional groups are
274 comprised of taxonomically diverse species of microbes with different lifestyle
275 strategies that are unlikely to share a conserved response to an ecosystem disturbance
276 (Ho *et al.*, 2012). While functional profiling allows us to measure the net response of
277 a functional group, and could serve as a proxy for determining the importance of the
278 group in a sample, it does not identify how specific organisms benefit from a
279 catalyzed transformation. Here we used a controlled microcosm experiment to
280 measure the response of soil communities to a disturbance in the form of changes in
281 moisture and nitrogen (urea) deposition. Functional analysis (qPCR) demonstrated a
282 biological response to urea, but differing responses to moisture depending on group
283 (Fig. 1). Responses are potentially linked to different life strategies amongst these
284 groups. Ammonia oxidizers displayed contrasting population and expression profiles,

285 suggesting niche differentiation driven by time and/or substrate concentration. AOA
286 responded early, and declined, as new N was made available while AOB responded
287 later with population swings spanning from near detection limit to most dominant
288 group. These observations match prior reports showing AOA prefer low N conditions,
289 while AOB respond vigorously to N deposition (Di *et al.*, 2010; Sterngren *et al.*,
290 2015). This has been interpreted as evidence for differing lifestyles for AOB and
291 AOA, with AOA preferring nutrient poor conditions and AOB dominating in rich
292 ones (Sterngren *et al.*, 2015). However, prior assertions that AOB are solely important
293 for driving nitrification might be overstated given that transcriptional activity for both
294 groups is comparable if compared at peak time (Di *et al.*, 2009). This contrasting use
295 of energy between functionally redundant organisms might explain the low
296 correlations between processes and the abundance of their respective functional
297 populations (Rocca *et al.*, 2015). When we examine the response of other functional
298 groups benefiting from influxes of N, like denitrifiers, we see no significant change in
299 population sizes suggesting that either energy is being utilized for maintenance or
300 otherwise for redox balance (Hartsock and Shapleigh, 2011; Y. Li *et al.*, 2012;
301 Dietrich *et al.*, 2013). In contrast, organism which benefit from low levels of N, like N
302 fixers, decline in response to exogenous N demonstrating real time selective pressure
303 in a complex ecosystem. These responses also highlight the temporal nature of these
304 relationships and how by following niche differentiation high number of functionally
305 redundant organisms can be maintained (Stempfhuber *et al.*, 2016).

306 Observations also highlight how lifestyle preferences for organisms are
307 reflected in their dominance in the ecosystem. Prior work suggests that AOA
308 dominate in soils with low N inputs, but AOB numbers are higher at times of high N
309 loading or in ecosystems with consistent N deposition (Gong *et al.*, 2013; Sterngren *et*

310 *al.*, 2015; Venterea *et al.*, 2015; C. Li *et al.*, 2016). This would suggest that a dynamic
311 ecosystem with varying nutrient levels would select for a higher diversity of
312 organisms that maintain ecosystem processes stable over time and space (Wang and
313 Loreau, 2014). Indeed, our data supports this with alpha diversity (calculated based on
314 16S amplicon analysis at both DNA and RNA) decreasing in response to urea. This is
315 inconsistent with plant responses to nutrient deposition in which multiple resources
316 need to be added to elicit a response (Harpole *et al.*, 2016), although contrasting
317 results have been observed (Suding *et al.*, 2005; Bai *et al.*, 2010; Song *et al.*, 2011;
318 2012). For microbes, high site to site variance is reported (De Schrijver *et al.*, 2011;
319 Leff *et al.*, 2015), but similar negative responses are suggested and could be linked to
320 increased competition in the absence of natural ecosystem variability. However, links
321 between microbial and plant response suggest interplay between the response of
322 macro and microbiota (Zeng *et al.*, 2016). While previous work suggests an important
323 role for moisture in controlling community composition (Waldrop and Firestone,
324 2006), we only observed a modifier role in our experiment.

325 Although broad observations align with ecological theory, precise
326 identification of responsive organisms is rarely carried out. Here we note that while at
327 phylum level clear responses (+/- fold change) are observed, variance is seen at the
328 OTU level suggesting the intra-taxonomic diversity we hypothesized reflected the life
329 history strategies of the organisms. Attempts to link specific transformations to
330 organisms failed, potentially due to the succession of functionally redundant
331 organisms that respond at different time with non-overlapping optima. That is, while
332 functional gene abundance provides the population size of organisms capable of
333 carrying out a process, the group may be composed of many OTUs with divergent life
334 strategies or metabolic potentials that affect when they can respond. This makes

335 functional gene measurement an average of all OTU subpopulations carrying that
336 gene. However, community response allows us to identify OTUs responsive to N
337 deposition, which when analyzed independently, provides insights into metabolic
338 preferences (i.e. aerobic vs. anaerobic, nitrifier vs. denitrifier) based on time and
339 response to treatments. Taxonomic groups regularly recognized as native to, or
340 abundant in, oligotrophic conditions declined in the presence of urea. Most of these
341 groups are still poorly understood, and included the Acidobacteria, Verrucomicrobia,
342 Nitrospirae, Candidate Division WS3 (also referred to as candidate phylum
343 Latescibacteria) and the Thaumarchaeota. These organisms are predicted to be slow
344 growers with the Thaumarchaeal response confirming the AOA patterns observed at
345 the functional level. In contrast, positively responding organisms are those generally
346 associated with groups considered eutrophic or capable of fast response. This
347 discrepancy based on life history strategies has been proposed and applied to
348 microbes previously, and suggests that an organisms' ability to grow, utilize carbon,
349 generate proteins and efficiently transform resources to biomass, amongst others, is
350 related to its rRNA operon copy number (Klappenbach *et al.*, 2000; Stevenson and
351 Schmidt, 2004; Dethlefsen and Schmidt, 2007; Roller *et al.*, 2016). When applied to
352 communities, it is associated with microbial successions in which decreases in copy
353 numbers are associated with later stages of succession including in soils (Nemergut *et*
354 *al.*, 2015). For example, two OTUs matching the Verrucomicrobial OTU DA101
355 where found to be negatively affected by urea, and at least one was found to be highly
356 abundant under background conditions. DA101 seems to be a common soil (and
357 grassland) organism identified throughout the world (Felske and Akkermans, 1998;
358 O'Farrell and Janssen, 1999; Brewer *et al.*, 2016). Based on growth (Sangwan *et al.*,
359 2005) and genome reconstructions (Brewer *et al.*, 2016), these organisms are

360 predicted to be slow but efficient growers (k strategists). In contrast, most of the
361 positively affected organisms seemed to posses higher rrn copy numbers and included
362 members of the Proteobacteria and Bacteroidetes in line with prior predictions (Fierer
363 *et al.*, 2007). Statistical analysis supported this interpretation with low copy numbers
364 (1-2) significantly associated to a negative response to N deposition, while high copy
365 numbers (>2) were linked to increased capacity for growth, growth rate and maximum
366 abundance. However, we found a non-linear relationship between increased rrn copy
367 numbers and growth capacity, best fitted by models reaching an asymptote (like
368 Michaelis-Menten and logistic curves). These are first order models that suggest that
369 while a benefit exists where increased copy numbers lead to increased growth rate,
370 after a certain threshold other variables might limit any benefit. Alternatively, a
371 decrease in growth rate might be observed with increasing copy numbers once a
372 tradeoff threshold is passed (Lipson, 2015). However, when rrn copy numbers are
373 log2 transformed, a significant linear fit was observed as seen in prior studies (Roller
374 *et al.*, 2016). In our study these predictions are made complicated due to the observed
375 intra-taxonomic variance that can arise from the lack of accurate knowledge of copy
376 numbers for many organisms, or from metabolic plasticity at higher taxonomic levels.
377 In addition, our analysis focused on N responsive organisms only, and with only 38
378 identified it indicates that most organisms were neither positively nor negatively
379 affected. This could explain why certain organisms (e.g. Actinobacteria) expected to
380 be k strategist, based on their ability to produce secondary metabolites (Abdelmohsen
381 *et al.*, 2015) and compete with other organisms (Barka *et al.*, 2015), showed a positive
382 response to N deposition. Alternatively, the low number of responsive organisms
383 could indicate that our false discovery rate corrections were too restrictive.

384 These findings help us get closer to understanding not just the metabolic
385 potential of organisms in soils, but the role specific pathways play for an organism. It
386 also allows us to understand the repercussion of disturbances and management of soils
387 on below ground biodiversity. The knowledge gained through these type of
388 observations, and integration of life history strategies into microbial ecology, will get
389 us one step closer to microbiome management as part of soil care.

390 **Experimental procedure**

391 *Sample collection and experimental design*

392 A detailed methodology can be found in (Clough *et. al*., In review). In brief,
393 soil (8°15'W, 52°9'N) was collected from a permanently grazed agricultural grassland
394 (dairy pasture) in March (early spring) at the Teagasc Moorepark Research Centre,
395 County Cork, Ireland. Soil was sampled after the turf was removed and a spade was
396 used to randomly sample the A-horizon (5-20 cm depth, excluding grass layer). To
397 avoid fresh N loading, fields had not been grazed for over a month. Field moist
398 samples were immediately shipped to Lincoln University, New Zealand and kept at
399 4°C until processed. Prior to use, soil was sieved (≤ 2 mm) to remove any stones,
400 plant roots or earthworms and packed into stainless steel rings (7.3 cm internal
401 diameter, 7.4 cm deep) to a depth of 4.1 cm at *in situ* soil bulk density (1.1 Mg m^{-3}
402 with a gravimetric water content (θg) of 0.24 g water g^{-1} soil). The resulting cores had
403 a total porosity of 0.58 cm^{3} pores cm^{-3} soil and were arranged in a factorial
404 experiment replicated four times. Soil cores were maintained at two moisture
405 contents: high (near saturated; -1.0 kPa) and low (field capacity; -10 kPa) moisture
406 using tension tables (Romano et al., 2002). These moisture contents, -1 and -10 kPa
407 respectively, corresponded to 53% and 30% volumetric water content, or 91% and

408 52% water-filled pore space (WFPS). Nitrogen was applied as a urea solution at 2141
409 kg urea/ha dry soil (equivalent to a single urination event at the higher rate expected
410 under bovine urine deposition of 1000 kg N ha^{-1}). Four treatments in total were
411 carried out (replicated four times each for a total of 112 cores analyzed) representing
412 two levels of urea and two levels of moisture: urea + high moisture (HM +N; Urea _-
413 1.0kPa), urea + low moisture (LM +N; Urea _-10kPa), no urea + high moisture (HM
414 –N; No Urea _-1.0kPa) and no urea + low moisture (LM –N; No Urea_-10kPa). All
415 cores where held at 20°C for a period of 63 days.
416

417 *Soil pH, and inorganic-N measurements*

418 Soil pH was monitored throughout the experiment using a flat surface pH
419 electrode (Broadley James Corp., Irvine, California). Inorganic N concentrations
420 (NH_4^+, NO_2^-, NO_3^-) were determined by destructively sampling batches of soil cores.
421 Each core was homogenized and a subsample was extracted (10 g dry soil: 100 ml 2M
422 KCl shaken for 1 hour), filtered (Whatman 42) and analyzed using flow injection
423 analysis (Blakemore et al., 1987). N_2O flux was determined by placing a soil core into
424 a 1-L stainless steel tin fitted with a gas-tight lid and rubber septa. The headspace was
425 sampled after 15 and 30 minutes and analyzed using an automated gas chromatograph
426 (8610; SRI Instruments, Torrance, CA), linked to an autosampler (Gilson 222XL;
427 Gilson, Middleton, WI) as previously described (Clough et al., 2006).

428 *Nucleic acids extraction*

429 Samples for RNA and DNA extraction were collected simultaneously with
430 samples for inorganic N analysis, but only samples at 0, 7, 14, 21, 35, 63 days were
431 processed for nucleic acids. Each biological replicate was extracted and analyzed

432 separately. For each extraction 2 g (wet weight) of soil were processed using the
433 PowerSoil Total RNA Isolation and DNA Elution Accessory Kits (MoBio, Carlsbad,
434 CA) as per manufacturer's instructions, with slight modifications. Bead beating was
435 done in a Geno/Grinder 2010 (SPEX SamplePrep, LLC, Metuchen, NJ) using two
436 rounds of beating (1750 strokes/min) for 15 s with a 1 min pause in between. The
437 total elution volume for RNA and DNA was 60 µl and 100 µl respectively. RNA was
438 treated with DNase I (RNase-Free) (New England Biolabs, USA) as per the
439 manufacturer's protocol. RNA quality was assessed by denaturing gel electrophoresis.
440 RNA and DNA concentration, purity and humic acid contamination were determined
441 using a Nanodrop Spectrophotometer, ND-1000 (Thermo Scientific). All extractions
442 were stored at -80 ^{o}C until downstream analyses.

443

444 *Reverse transcription (RT)*

445 Triplicate cDNA conversions (technical replicates) were performed for each
446 RNA extraction using the Maxima H Minus First Strand cDNA Synthesis Kit
447 (Thermo Scientific) according to manufacturer's protocol. Each 20 µl reaction
448 contained: 13 µl of RNA (208 ng Total RNA), 1 µl of random hexamers (100 pmol),
449 1µl of dNTP mix (0.5 mM final conc.) and 5 µl of master mix (4 µl of 5X RT buffer
450 and 1 µl Maxima H Minus reverse transcriptase). All technical replicates for a sample
451 were combined and stored at -80°C until further analysis. All further analyses were
452 performed on the same cDNA pool for each sample.

453

454 *16S rRNA gene amplicon sequencing*

455 16S rRNA gene amplicon sequencing was performed using primers
456 515F/806R (V4 region of the 16S gene) and the Earth Microbiome Project conditions
457 (Version 4_13) (Caporaso *et al.*, 2012). All samples were run simultaneously on a
458 single Illumina MiSeq run. Sequences were first processed in Qiime (version 1.9.1)
459 using default parameters (Caporaso *et al.*, 2010). Sequences were clustered into
460 Operational Taxonomic Units (OTUs) at 97% sequence similarity using the SILVA
461 (version 119) reference library (Quast *et al.*, 2012) and UCLUST (Edgar, 2010)
462 following the open-reference Operational Taxonomic Unit (OTU) picking protocol.
463 Taxonomic identification was done using BLAST against the SILVA database (max-e
464 value = 0.001) (Altschul *et al.*, 1990). Subsampling and rarefactions (10 times) were
465 performed to equal read depths of 7,400 per sample, and samples below that threshold
466 were removed. After rarefaction, all 10 OTU tables were merged and exported for
467 further processing in R (R Development Core Team, 2008). The 16S amplicon
468 sequences are available in the NCBI SRA database (accession numbers SRP091980).

469

470 *Quantification of gene and transcript abundance*

471 Quantitative PCR (qPCR) was performed in 384-well plates using the ViiA7
472 real-time PCR system (Applied Biosystems, Carlsbad, CA). Absolute quantification
473 was done using a 10-fold serial dilution (10^8 to 10^1) of known copy numbers of
474 pGEM-T easy (Promega, Madison, Wisconsin, USA) cloned templates as standards.
475 For all targets qPCR runs included cloned standards, no template control and no
476 reverse transcription controls (RNA) run in triplicate. No inhibition or positive
477 amplification on negative controls was observed for any target. All DNA and cDNA
478 samples were run in quadruplicates to determine abundance of: prokaryotes (16S

479 rRNA gene), ammonia oxidizers (archaeal [AOA] & bacterial [AOB] ammonia
480 monooxygenase gene; *amoA*), denitrifiers (cytochrome cd1-type nitrite reductase
481 gene; *nirS,* and Clade I nitrous oxide reductase gene; *nosZI*) and nitrogen fixers
482 (nitrogenase gene; *nifH*).

483 All reactions were performed in 10 µl volumes containing: 1× Master Mix
484 (Fast SYBR Green Master Mix, ABI), 0.2-0.6 µM of each primer [0.2 µM for AOA
485 (Tourna *et al.*, 2008), 0.6 µM for AOB (Avrahami *et al.*, 2003); 0.5 µM for 16S
486 rRNA (Hartman *et al.*, 2009); *nirS* (Throbäck *et al.*, 2004; Yergeau *et al.*, 2007),
487 *nosZI* (Henry *et al.*, 2006) & *nifH* (Rösch and Bothe, 2005)], 2 µl of template [DNA
488 (1 ng total) or cDNA (80× diluted RT reaction, i.e. total 0.13 ng RNA)] and
489 autoclaved Milli-Q H_2O to a final volume of 10 µl. Primers and qPCR conditions are
490 summarized in Table S1. A melt curve analysis (95°C for 15 s, 60°C for 1 min then
491 increasing 0.05^{o}C/s (data acquisition) until 95°C) was performed to test for specificity
492 and to confirm no amplification in the negative controls.

493

494 *Statistical analyses*

495 All statistical analyses were performed in R (R Development Core Team,
496 2008) using the phyloseq (McMurdie and Holmes, 2013), pvclust (Suzuki and
497 Shimodaira, 2006), vegan (Oksanen *et al.*) and mpmcorrelogram packages. Detailed
498 descriptions can be found in supplemental methods.

499

500 *Growth rate estimation and prediction of rRNA operon (rrn) copy numbers*

501 rrn copy numbers for identified OTUs were predicted using the ribosomal RNA
502 operon copy number database (rrnDB) (Stoddard *et al.*, 2015). For each OTU,

503 information from the closest strain available was selected. In instances where a
504 closely related organism was not available, the mean copy number for the closest
505 taxonomic group (i.e. genus, class, etc.) was used. Copy numbers where then
506 compared to the maximum observed abundance and the maximum observed fold
507 change (calculated based on lowest observed abundance for the same organism in a
508 preceding time point for OTUs showing growth or succeeding time points for those
509 decreasing in abundance). An estimated growth rate was calculated for OTUs
510 showing increases in population size in response to N using the following formula:

511 $$N_t = N_0 * e^{rt}$$

512

513 where: N_t: The amount at time t; N_0: The amount at time 0; r: exponential growth rate;
514 t: Time passed

515 *Fit model for rrn copy numbers*

516 Both non-linear (Michaelis-Menten) and linear regressions were used to fit rrn
517 copy numbers and population changes (i.e. maximum abundance and fold-change),
518 and growth rate (per day). The fit model was performed in R using "drc" and
519 "ggplot2" packages.

520

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527 **References**

528 Abdelmohsen, U.R., Grkovic, T., Balasubramanian, S., Kamel, M.S., Quinn, R.J., and
529 Hentschel, U. (2015) Elicitation of secondary metabolism in actinomycetes.
530 *Biotechnology Advances* **33**: 798–811.
531 Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990) Basic
532 local alignment search tool. *J Mol Biol* **215**: 403–410.
533 Avrahami, S., Liesack, W., and Conrad, R. (2003) Effects of temperature and
534 fertilizer on activity and community structure of soil ammonia oxidizers. *Environ*
535 *Microbiol* **5**: 691–705.
536 Bai, Y., Wu, J., Clark, C.M., Naeem, S., and Pan, Q. (2010) Tradeoffs and thresholds
537 in the effects of nitrogen addition on biodiversity and ecosystem functioning:
538 evidence from inner Mongolia Grasslands. *Glob Change Biol* **16**: 358–372.
539 Baral, K.R., Thomsen, A.G., Olesen, J.E., and Petersen, S.O. (2014) Controls of
540 nitrous oxide emission after simulated cattle urine deposition. *Agr Ecosyst*
541 *Environ* **188**: 103–110.
542 Barka, E.A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H.P., et
543 al. (2015) Taxonomy, Physiology, and Natural Products of Actinobacteria.
544 *Microbiol Mol Biol R* **80**: 1–43.
545 Bayer, B., Vojvoda, J., Offre, P., Alves, R.J.E., Elisabeth, N.H., Garcia, J.A., et al.
546 (2015) Physiological and genomic characterization of two novel marine
547 thaumarchaeal strains indicates niche differentiation. **10**: 1051–1063.
548 Brenzinger, K., Dörsch, P., and Braker, G. (2015) pH-driven shifts in overall and
549 transcriptionally active denitrifiers control gaseous product stoichiometry in
550 growth experiments with extracted bacteria from soil. *Front Microbiol* **6**: 1226.
551 Brewer, T., Handley, K., Carini, P., Gibert, J., and Fierer, N. (2016) Genome
552 reduction in an abundant and ubiquitous soil bacterial lineage.
553 Butterbach-Bahl, K., Baggs, E.M., Dannenmann, M., Kiese, R., and Zechmeister-
554 Boltenstern, S. (2013) Nitrous oxide emissions from soils: how well do we
555 understand the processes and their controls? *Phil Trans R Soc B* **368**: 20130122–
556 20130122.
557 Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello,
558 E.K., et al. (2010) QIIME allows analysis of high-throughput community
559 sequencing data. *Nat Meth* **7**: 335–336.
560 Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N.,
561 et al. (2012) Ultra-high-throughput microbial community analysis on the Illumina
562 HiSeq and MiSeq platforms. *ISME J* **6**: 1621–1624.
563 Clough, T.J., Lanigan, G.J., de Klein, C.A.M., Samad, M.S., Morales, S.E., Rax, D.,
564 et al. (n.d.). Influence of soil moisture on co-denitrification fluxes from a urea-
565 affected pasture soil. In Review.
566 de Klein, C.A., Shepherd, M.A., and van der Weerden, T.J. (2014) Nitrous oxide
567 emissions from grazed grasslands: interactions between the N cycle and climate
568 change — a New Zealand case study. *Curr Opin Environ Sustain* **9-10**: 131–139.
569 de Klein, C.A.M., Luo, J., Woodward, K.B., Styles, T., Wise, B., Lindsey, S., and
570 Cox, N. (2014) The effect of nitrogen concentration in synthetic cattle urine on
571 nitrous oxide emissions. *Agr Ecosyst Environ* **188**: 85–92.
572 De Schrijver, A., De Frenne, P., Ampoorter, E., Van Nevel, L., Demey, A., Wuyts,
573 K., and Verheyen, K. (2011) Cumulative nitrogen input drives species loss in
574 terrestrial ecosystems. *Global Ecology and Biogeography* **20**: 803–816.
575 Delmont, T.O., Eren, A.M., Maccario, L., Prestat, E., Esen, Ö.C., Pelletier, E., et al.

576 (2015) Reconstructing rare soil microbial genomes using in situ enrichments and
577 metagenomics. *Front Microbiol* **6**: 1–15.
578 Dethlefsen, L. and Schmidt, T.M. (2007) Performance of the translational apparatus
579 varies with the ecological strategies of bacteria. *J Bacteriol* **189**: 3237–3245.
580 Di, H.J., Cameron, K.C., Shen, J.-P., Winefield, C.S., O'Callaghan, M., Bowatte, S.,
581 and He, J.-Z. (2010) Ammonia-oxidizing bacteria and archaea grow under
582 contrasting soil nitrogen conditions. *Fems Microbiol Ecol* **72**: 386–394.
583 Di, H.J., Cameron, K.C., Shen, J.P., Winefield, C.S., O'Callaghan, M., Bowatte, S.,
584 and He, J.Z. (2009) Nitrification driven by bacteria and not archaea in nitrogen-
585 rich grassland soils. *Nature Geoscience* **2**: 621–624.
586 Dietrich, L.E.P., Okegbe, C., Price-Whelan, A., Sakhtah, H., Hunter, R.C., and
587 Newman, D.K. (2013) Bacterial Community Morphogenesis Is Intimately Linked
588 to the Intracellular Redox State. *J Bacteriol* **195**: 1371–1380.
589 Edgar, R.C. (2010) Search and clustering orders of magnitude faster than BLAST.
590 *Bioinformatics* **26**: 2460–2461.
591 Felske and Akkermans (1998) Prominent occurrence of ribosomes from an uncultured
592 bacterium of the Verrucomicrobiales cluster in grassland soils. *Letters in Applied*
593 *Microbiology* **26**: 219–223.
594 Fierer, N., Bradford, M.A., and Jackson, R.B. (2007) Toward an ecological
595 classification of soil bacteria. *Ecology* **88**: 1354–1364.
596 Gong, P., Zhang, L., Wu, Z., Shang, Z., and Li, D. (2013) Does the nitri cation
597 inhibitor dicyandiamide affect the abundance of ammonia-oxidizing bacteria and
598 archaea in a Hap-Udic Luvisol? *J Soil Sci Plant Nutr* **13**: 35–42.
599 Gubry-Rangin, C., Kratsch, C., Williams, T.A., McHardy, A.C., Embley, T.M.,
600 Prosser, J.I., and Macqueen, D.J. (2015) Coupling of diversification and pH
601 adaptation during the evolution of terrestrial Thaumarchaeota. *Proc Nat Acad Sci*
602 **112**: 9370–9375.
603 Hamonts, K., Balaine, N., Moltchanova, E., Beare, M., Thomas, S., Wakelin, S.A., et
604 al. (2013) Influence of soil bulk density and matric potential on microbial
605 dynamics, inorganic N transformations, N_2O and N_2 fluxes following urea
606 deposition. *Soil Biol Biochem* **65**: 1–11.
607 Harpole, W.S., Sullivan, L.L., Lind, E.M., Firn, J., Adler, P.B., Borer, E.T., et al.
608 (2016) Addition of multiple limiting resources reduces grassland diversity. *Nature*
609 **537**: 93–96.
610 Hartman, A.L., Lough, D.M., Barupal, D.K., Fiehn, O., Fishbein, T., Zasloff, M., and
611 Eisen, J.A. (2009) Human gut microbiome adopts an alternative state following
612 small bowel transplantation. *Proc Nat Acad Sci* **106**: 17187–17192.
613 Hartsock, A. and Shapleigh, J.P. (2011) Physiological Roles for Two Periplasmic
614 Nitrate Reductases in Rhodobacter sphaeroides 2.4.3 (ATCC 17025). *J Bacteriol*
615 **193**: 6483–6489.
616 Hatzenpichler, R. (2012) Diversity, physiology, and niche differentiation of ammonia-
617 oxidizing archaea. *Appl Environ Microb* **78**: 7501–7510.
618 Henry, S., Bru, D., Stres, B., Hallet, S., and Philippot, L. (2006) Quantitative
619 detection of the *nosZ* gene, encoding nitrous oxide reductase, and comparison of
620 the abundances of 16S rRNA, *narG*, *nirK*, and *nosZ* genes in soils. *Appl Environ*
621 *Microb* **72**: 5181–5189.
622 Herrero, M., Henderson, B., Havlík, P., Thornton, P.K., Conant, R.T., Smith, P., et al.
623 (2016) Greenhouse gas mitigation potentials in the livestock sector. *Nature*
624 *Climate Change* **6**: 452–461.
625 Ho, A., Kerckhof, F.-M., Luke, C., Reim, A., Krause, S., Boon, N., and Bodelier,

P.L.E. (2012) Conceptualizing functional traits and ecological characteristics of methane-oxidizing bacteria as life strategies. *Environmental Microbiology Reports* **5**: 335–345.

Jia, Z. and Conrad, R. (2009) Bacteriarather than Archaea dominate microbial ammonia oxidation in an agricultural soil. *Environ Microbiol* **11**: 1658–1671.

Klappenbach, J.A., Dunbar, J.M., and Schmidt, T.M. (2000) rRNA Operon Copy Number Reflects Ecological Strategies of Bacteria. *Appl Environ Microb* **66**: 1328–1333.

Leff, J.W., Jones, S.E., Prober, S.M., Barberán, A., Borer, E.T., Firn, J.L., et al. (2015) Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proc Nat Acad Sci* **112**: 10967–10972.

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W., et al. (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* **442**: 806–809.

Li, C., Di, H.J., Cameron, K.C., Podolyan, A., and Zhu, B. (2016) Effect of different land use and land use change on ammonia oxidiser abundance and N_2O emissions. *Soil Biol Biochem* **96**: 169–175.

Li, Y., Katzmann, E., Borg, S., and Schuler, D. (2012) The Periplasmic Nitrate Reductase Nap Is Required for Anaerobic Growth and Involved in Redox Control of Magnetite Biomineralization in Magnetospirillum gryphiswaldense. *J Bacteriol* **194**: 4847–4856.

Lipson, D.A. (2015) The complex relationship between microbial growth rate and yield and its implications for ecosystem processes. *Front Microbiol* **6**: 1–5.

Liu, B., Liu, B., Frostegard, Å., Frostegård, Å., Bakken, L.R., and Bakken, L.R. (2014) Impaired Reduction of N_2O to N_2 in Acid Soils Is Due to a Posttranscriptional Interference with the Expression of nosZ. *mBio* **5**: e01383–14–e01383–14.

Liu, B., Mørkved, P.T., Frostegård, Å., and Bakken, L.R. (2010) Denitrification gene pools, transcription and kinetics of NO, N_2O and N_2 production as affected by soil pH. *Fems Microbiol Ecol* **72**: 407–417.

McMurdie, P.J. and Holmes, S. (2013) phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE*.

Morales, S.E. and Holben, W.E. (2013) Functional response of a near-surface soil microbial community to a simulated underground CO_2 storage leak. *PLoS ONE* **8**: e81742–10.

Nemergut, D.R., Knelman, J.E., Ferrenberg, S., Bilinski, T., Melbourne, B., Jiang, L., et al. (2015) Decreases in average bacterial community rRNA operon copy number during succession. **10**: 1147–1156.

Nicol, G.W., Leininger, S., Schleper, C., and Prosser, J.I. (2008) The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. *Environ Microbiol* **10**: 2966–2978.

Oksanen, J., Blanchet, F.G., Kindt, R., and Legendre, P. Vegan: Community Ecology Package (2013) R package version 2.0-7. https://cran.r-project.org/package=vegan.

O'Farrell, K.A. and Janssen, P.H. (1999) Detection of Verrucomicrobia in a Pasture Soil by PCR-Mediated Amplification of 16S rRNA Genes. *Appl Environ Microb* **65**: 4280–4284.

Pratscher, J., Dumont, M.G., and Conrad, R. (2011) Ammonia oxidation coupled to CO_2 fixation by archaea and bacteria in an agricultural soil. pp. 4170–4175.

Prosser, J.I. and Nicol, G.W. (2012) Archaeal and bacterial ammonia-oxidisers in soil:

676 the quest for niche specialisation and differentiation. *Trends in Microbiology* **20**:
677 523–531.
678 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2012)
679 The SILVA ribosomal RNA gene database project: improved data processing and
680 web-based tools. *Nucleic Acid Res* **41**: D590–D596.
681 R Development Core Team (2008) R: A language and environment for statistical
682 computing. *R Foundation for Statistical Computing, Vienna, Austria*.
683 Rinke, C., Schwientek, P., Sczyrba, A., Ivanova, N.N., Anderson, I.J., Cheng, J.F., et
684 al. (2013) Insights into the phylogeny and coding potential of microbial dark
685 matter. *Nature* **499**: 431–437.
686 Rocca, J.D., Hall, E.K., Lennon, J.T., Evans, S.E., Waldrop, M.P., Cotner, J.B., et al.
687 (2015) Relationships between protein-encoding gene abundance and
688 corresponding process are commonly assumed yet rarely observed. **9**: 1693–1699.
689 Roller, B.R.K., Stoddard, S.F., and Schmidt, T.M. (2016) Exploiting rRNA operon
690 copy number to investigate bacterial reproductive strategies. *Nature Microbiology*
691 1–7.
692 Rösch, C. and Bothe, H. (2005) Improved assessment of denitrifying, N_2-fixing, and
693 total-community bacteria by terminal restriction fragment length polymorphism
694 analysis using multiple restriction enzymes. *Appl Environ Microb* **71**: 2026–2035.
695 Saggar, S., Jha, N., Deslippe, J., Bolan, N.S., Luo, J., Giltrap, D.L., et al. (2013)
696 Denitrification and N_2O:N_2 production in temperate grasslands: Processes,
697 measurements, modelling and mitigating negative impacts. *Sci Total Environ* **465**:
698 173–195.
699 Sangwan, P., Kovac, S., Davis, K.E.R., Sait, M., and Janssen, P.H. (2005) Detection
700 and cultivation of soil verrucomicrobia. *Appl Environ Microb* **71**: 8402–8410.
701 Simon, J. and Klotz, M.G. (2013) Diversity and evolution of bioenergetic systems
702 involved in microbial nitrogen compound transformations. *Biochimica et*
703 *Biophysica Acta (BBA)-Bioenergetics* **1827**: 114–135.
704 Song, L., Bao, X., Liu, X., and Zhang, F. (2012) Impact of nitrogen addition on plant
705 community in a semi-arid temperate steppe in China. *Journal of Arid Land* **4**: 3–
706 10.
707 Song, L., Bao, X., Liu, X., Zhang, Y., Christie, P., Fangmeier, A., and Zhang, F.
708 (2011) Nitrogen enrichment enhances the dominance of grasses over forbs in a
709 temperate steppe ecosystem. *Biogeosciences* **8**: 2341–2350.
710 Spiro, S. (2012) Nitrous oxide production and consumption: regulation of gene
711 expression by gas-sensitive transcription factors. *Phil Trans R Soc B* **367**: 1213–
712 1225.
713 Stahl, D.A. and la Torre, de, J.R. (2012) Physiology and diversity of ammonia-
714 oxidizing archaea. *Annu. Rev. Microbiol.* **66**: 83–101.
715 Stempfhuber, B., Richter-Heitmann, T., Regan, K.M., Kölbl, A., Wüst, P.K., Marhan,
716 S., et al. (2016) Spatial Interaction of Archaeal Ammonia-Oxidizers and Nitrite-
717 Oxidizing Bacteria in an Unfertilized Grassland Soil. *Front Microbiol* **6**: 861–15.
718 Sterngren, A.E., Hallin, S., and Bengtson, P. (2015) Archaeal ammonia oxidizers
719 dominate in numbers, but bacteria drive gross nitrification in N-amended
720 grassland soil. *Front Microbiol* **6**: 1620–8.
721 Stevenson, B.S. and Schmidt, T.M. (2004) Life History Implications of rRNA Gene
722 Copy Number in Escherichia coli. *Appl Environ Microb* **70**: 6670–6677.
723 Stoddard, S.F., Smith, B.J., Hein, R., Roller, B.R.K., and Schmidt, T.M. (2015)
724 rrnDB: improved tools for interpreting rRNA gene abundance in bacteria and
725 archaea and a new foundation for future development. *Nucleic Acid Res* **43**:

D593–D598.
Suding, K.N., Collins, S.L., Gough, L., Clark, C., Cleland, E.E., Gross, K.L., et al. (2005) Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proc Nat Acad Sci* **102**: 4387–4392.
Suzuki, R. and Shimodaira, H. (2006) Pvclust: an R package for assessing the uncertainty in hierarchical clustering. *Bioinformatics* **22**: 1540–1542.
Taylor, A.E., Zeglin, L.H., Wanzek, T.A., Myrold, D.D., and Bottomley, P.J. (2012) Dynamics of ammonia-oxidizing archaea and bacteria populations and contributions to soil nitrification potentials. **6**: 2024–2032.
Throbäck, I.N., Enwall, K., Jarvis, Å., and Hallin, S. (2004) Reassessing PCR primers targeting *nirS*, *nirK* and *nosZ* genes for community surveys of denitrifying bacteria with DGGE. *Fems Microbiol Ecol* **49**: 401–417.
Tourna, M., Freitag, T.E., Nicol, G.W., and Prosser, J.I. (2008) Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms. *Environ Microbiol* **10**: 1357–1364.
Venterea, R.T., Clough, T.J., Coulter, J.A., Breuillin-Sessoms, F., Wang, P., Sadowsky, M.J., and A, R.P.L. (2015) Ammonium sorption and ammonia inhibition of nitrite-oxidizing bacteria explain contrasting soil N_2O production. *Sci. Rep.* **5**: 12153–15.
Waldrop, M.P. and Firestone, M.K. (2006) Response of microbial community composition and function to soil climate change. *Microb Ecol* **52**: 716–724.
Wei, W., Isobe, K., Nishizawa, T., Zhu, L., Shiratori, Y., Ohte, N., et al. (2015) Higher diversity and abundance of denitrifying microorganisms in environments than considered previously. 1–12.
Yergeau, E., Kang, S., He, Z., Zhou, J., and Kowalchuk, G.A. (2007) Functional microarray analysis of nitrogen and carbon cycling genes across an Antarctic latitudinal transect. *ISME J* **1**: 163–179.
Zeng, J., Liu, X., Song, L., Lin, X., Zhang, H., Shen, C., and Chu, H. (2016) Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. *Soil Biol Biochem* **92**: 41–49.

Figures

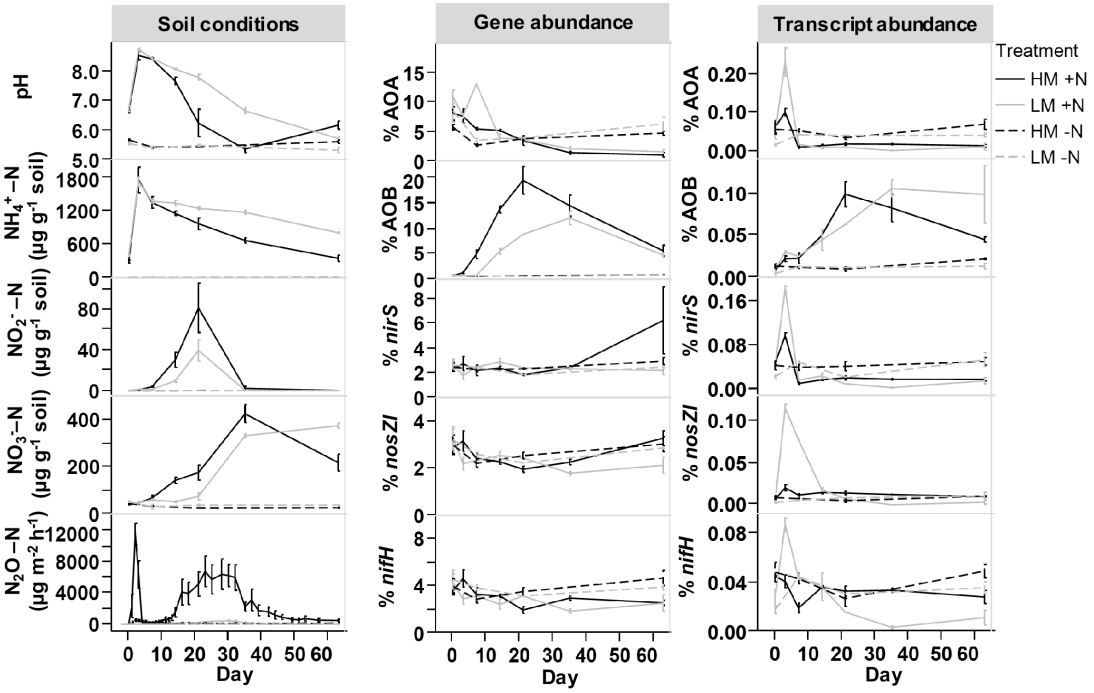

Fig. 1 Chemical transformations and biological (functional group) response in soils treated with urea (+/- 1000 µg N/g dry soil) under two moisture conditions (LM = low moisture [-10kPa]; HM = high moisture [-1.0kPa]). Error bars are the standard error of the mean (n ≥ 3, except gene abundance data of day 7 [n=1; LM soil] and day 21 [n=1; LM soil]) for replicate mesocosms. Gene and transcript abundance were measured by qPCR targeting: nitrifiers (AOA, ammonia oxidizing archaea; AOB, ammonia oxidizing bacteria), denitrifiers (*nirS,* cytochrome cd $_1$-containing nitrite reductase*; nosZI*, nitrous oxide reductase) and nitrogen fixers (*nifH*, nitrogenase reductase). All qPCR results are normalized to 16S rRNA copy numbers and presented as percent of the nucleic acid pool.

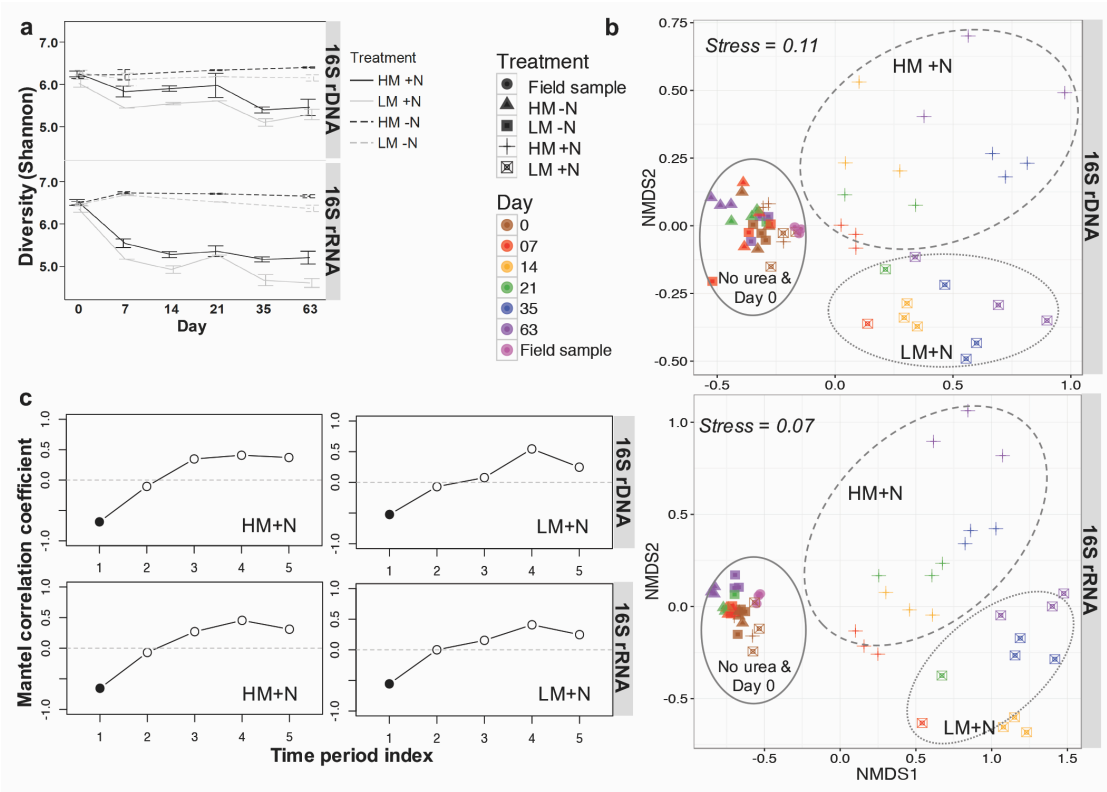

Fig. 2 Total microbial community response (based on 16S rRNA gene amplicon profiling and clustering of sequences at OTU level (97% sequence similarity)) to urea (+/-1000 µg N/g dry soil) under two moisture conditions (LM = low moisture [-10kPa]; HM = high moisture [-1.0kPa]) at both DNA and RNA level. Error bars are the standard error of the mean (n = 3, except day 7 [n=1; LM soil] and day 21 [n=1; LM soil]) for replicate mesocosms. (a) Changes in microbial diversity (Shannon) index over time in response to treatment. (b) Non-metric multidimensional scaling (NMDS) ordination plots based on Bray-Curtis distances showing relationships among samples based on OTU level changes in community composition. (c) Mantel correlogram showing autocorrelation on community composition by performing sequential Mantel tests between the Bray-Curtis dissimilarities and the grouping of samples using a time period index (index 1 represents 0-7 days; 2 represents 7-14; 3 represents 14-21; 4 represents 21-35; 5 represents 35-63). Filled circles represent

significant correlation ($p < 0.05$) in community composition at specific time periods, with open circles indicating no significant correlation.

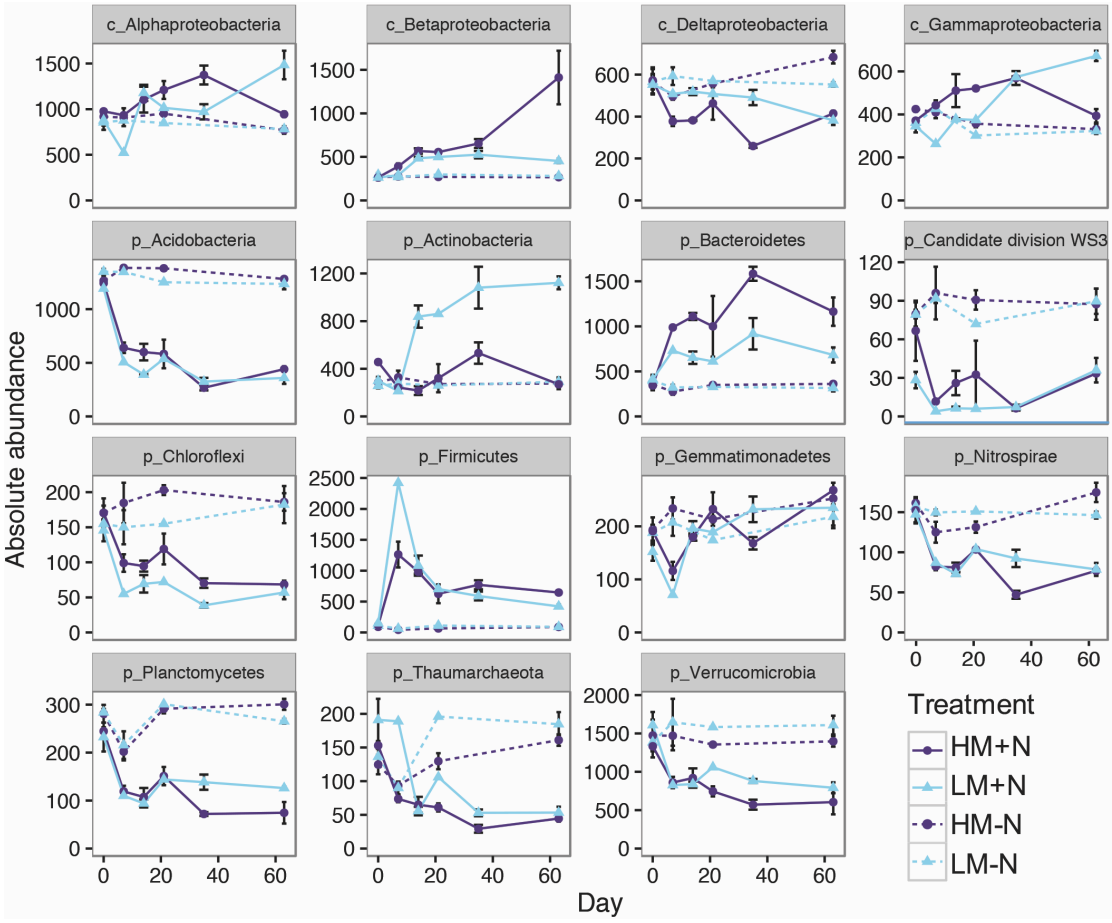

Fig. 3 Phylum and class level (for Proteobacteria only) changes in abundance (DNA) representing relative contribution >1% of all detected phyla (based on OTUs clustered at 97% sequence similarity). A total of 7,400 sequences were examined per sample. Error bars are the standard error of the mean (n = 3, except day 7 [n=1; LM soil] and day 21 [n=1; LM soil]) for replicate mesocosms. Treatments = +/- N [+/-1000 µg N/g dry soil] under two moisture conditions (LM = low moisture [-10kPa]; HM = high moisture [-1.0kPa]). Abbreviations: c: Class; p: Phylum. See supplemental Fig. S8 for relative abundance.

Fig. 4 Taxonomic summary of OTUs responsive to urea treatment identified through similarity percentages (SIMPER) analysis (representing top 50% cumulative sum). The 4 outer rings represent fold changes in response to urea under high and low moisture content (MH & LM respectively) at either DNA or RNA level, with blank gaps indicating OTUs not identified in SIMPER analysis under the specified ring condition. Nodes on the tree (moving outwards from center) correspond to taxonomic level [Domain, Phylum, Class, Order, Family, Genus and Species/OTUs]. Nodes are colored based on dominant response (>50% conserved fold change response across OTUs within a node) with black notes indicating equal representation of positive and

negatively responding OTUs. Shaded areas of branches delineate defined taxonomic groups. See Supplementary file (Table S3) for full classification.

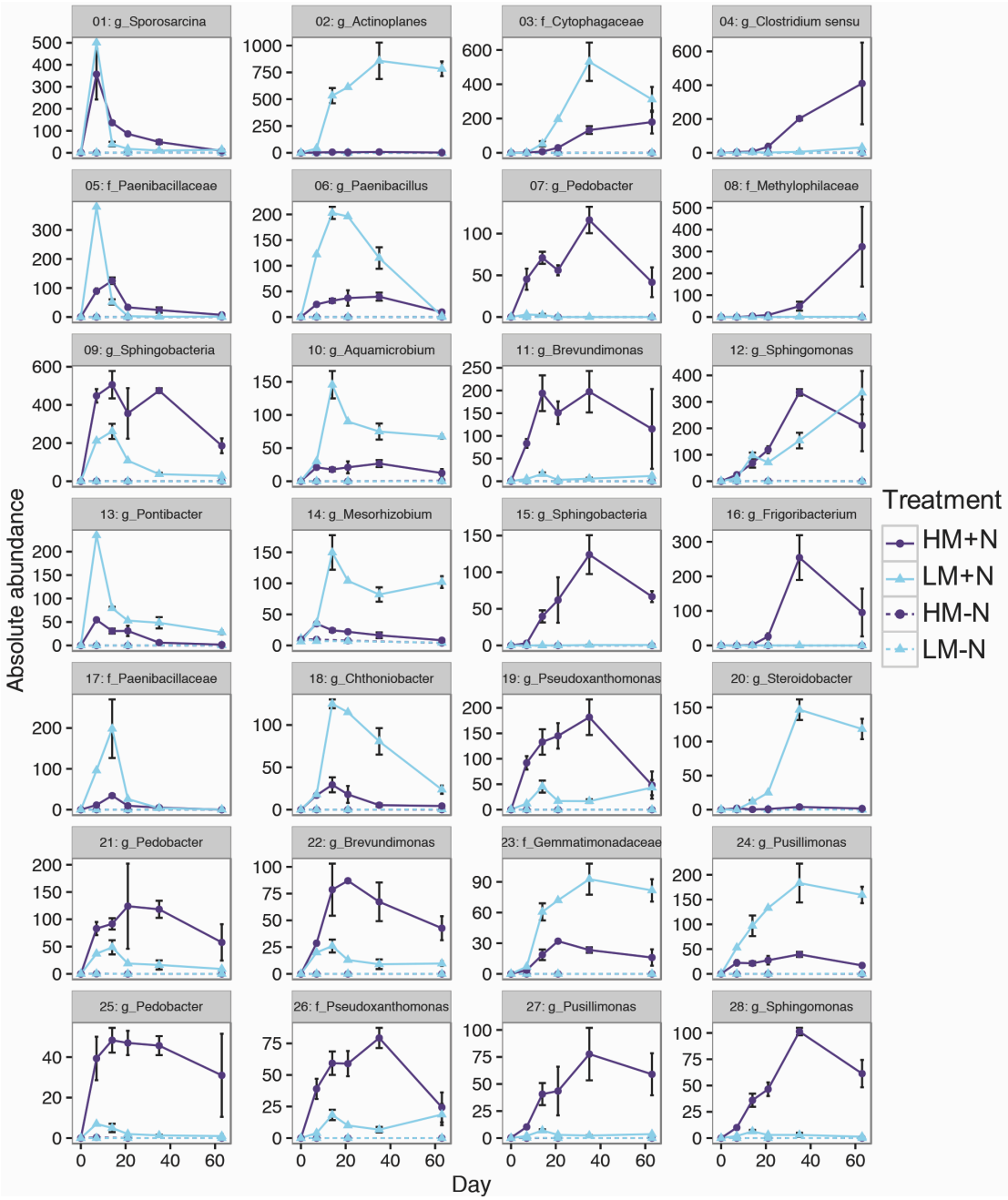

Fig. 5 Population (16S rDNA) changes (absolute abundance based on 7400 reads per samples) for OTUs identified as positively responsive to urea treatment based on similarity percentages (SIMPER) analysis (representing top 30% cumulative sum).

818 Treatments = +/- N [+/-1000 µg N/g dry soil] under two moisture conditions (LM =
819 low moisture [-10kPa]; HM = high moisture [-1.0kPa]).
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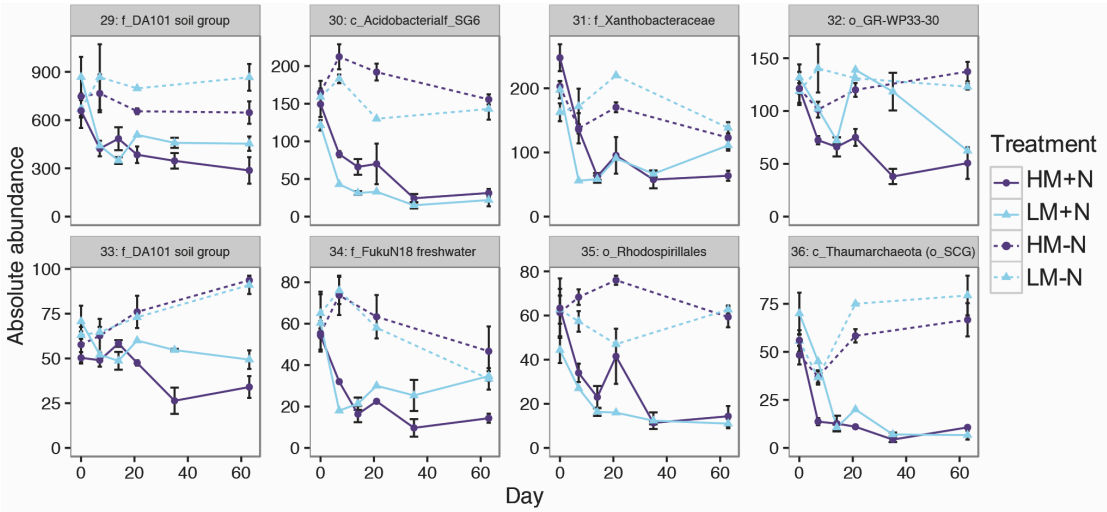

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824
825 **Fig. 6** Population (16S rDNA) changes (absolute abundance based on 7,400 reads per
826 samples) for OTUs identified as negatively responsive to urea treatment based on
827 similarity percentages (SIMPER) analysis (representing top 30% cumulative sum).
828 Treatments = +/- N [+/-1000 µg N/g dry soil] under two moisture conditions (LM =
829 low moisture [-10kPa]; HM = high moisture [-1.0kPa]).
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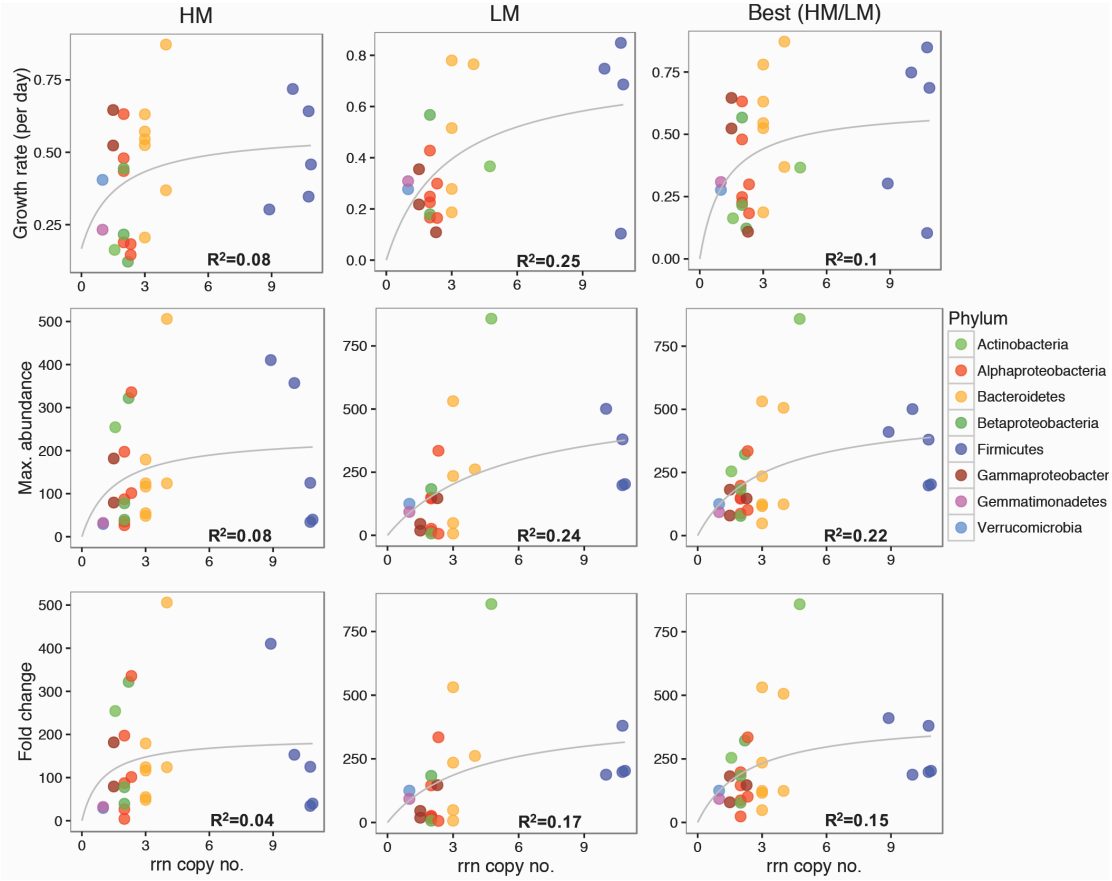

Fig. 7 Relationship between predicted ribosomal RNA operon (rrn) copy numbers and growth rate (per day), maximum observed population change, or fold change in response to N treatment under both high moisture (HM) content, low moisture (LM) content and best growth either in HM or in LM (based on maximum observed growth). Copy number was estimated using rrn database (Stoddard *et al.*, 2015). Copy number values were obtained by finding the closest match (lowest taxonomic level possible) to each OTU and retrieving the mean rRNA copy number for that group.