

1 **Comparing DNA, RNA and protein levels for measuring microbial activity in**
2 **nitrogen-amended soils**

3 Luis H. Orellana,^a Janet K. Hatt,^a Ramsunder Iyer,^{b,c} Karuna Chourey,^b Robert L. Hettich,^b Jim
4 C. Spain,^d Wendy H. Yang,^e Joanne C. Chee-Sanford,^f Robert A. Sanford,^g Frank E. Löffler,^{h,i}
5 Konstantinos T. Konstantinidis^{a,#}

6
7 ^a School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta,
8 Georgia, USA

9 ^b Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

10 ^c Graduate School of Genome Science and Technology, University of Tennessee, Knoxville,
11 Tennessee, USA

12 ^d Center for Environmental Diagnostics & Bioremediation, University of West Florida,
13 Pensacola, Florida, USA

14 ^e Department of Geology, University of Illinois at Urbana-Champaign, Urbana, Illinois USA

15 ^f U.S. Department of Agriculture, Agricultural Research Service, Urbana, Illinois, USA

16 ^g University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

17 ^h University of Tennessee, Knoxville, Tennessee, USA

18 ⁱ Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

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21 Running head: Multi-omics of soil microcosms

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23 [#]Address correspondence to Konstantinos T. Konstantinidis, kostas@ce.gatech.edu

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26 **ABSTRACT**

27 Multi-omic techniques can offer a comprehensive overview of microbial communities at the
28 gene, transcript and protein levels. However, to what extent these levels reflect *in situ* process
29 rates is less clear, especially in highly complex habitats such as soils. Here we performed
30 microcosm incubations using soil from a site with a history of agricultural management.
31 Microcosms, amended with isotopically labelled ammonium and urea to simulate a fertilization
32 event, showed nitrification (up to $4.1 \pm 0.87 \mu\text{g N-NO}_3^- \text{ g}^{-1} \text{ dry soil d}^{-1}$) and accumulation of N_2O
33 after 192 hours of incubation. Nitrification activity ($\text{NH}_4^+ \rightarrow \text{NH}_2\text{OH} \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^-$) was
34 accompanied by a 6-fold increase in relative expression of the 16S rRNA gene (RNA/DNA)
35 between 10 and 192 hours of incubation for ammonia-oxidizing bacteria (AOB) *Nitrosomonas*
36 and *Nitrosospira*. In contrast, ammonia-oxidizing archaea (AOA) and complete ammonia
37 oxidizer (comammox) nitrifiers showed stable gene expression during incubations but were
38 generally more abundant (DNA level) than their *Betaproteobacteria* AOB counterparts. A strong
39 relationship between nitrification activity and (mostly) betaproteobacterial ammonia
40 monooxygenase (*amoA*; $\text{NH}_4^+ \rightarrow \text{NH}_2\text{OH}$) and nitrite oxidoreductase (*nxrA*; $\text{NO}_2^- \rightarrow \text{NO}_3^-$)
41 transcript abundances revealed that mRNA levels quantitatively reflected measured activity and
42 were generally more sensitive than the DNA level in the microcosm incubations. Although
43 peptides related to housekeeping proteins from nitrite-oxidizing microorganisms were detected,
44 their abundance was not significantly correlated with activity, revealing that meta-proteomics
45 provided only a qualitative assessment of activity. Altogether, these findings underscore the
46 strengths and limitations of multi-omic approaches for assessing complex microbial
47 communities and provide the molecular means to assess nitrification processes in soils.

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52 **IMPORTANCE**

53 Even though the use of omic approaches has expanded our knowledge of the diversity of
54 microbial communities in natural and engineered systems, it is less clear how well the use of
55 whole community DNA-, RNA- or protein-based approaches reflect microbial activities. To this
56 end, we directly compared the different levels of molecular information (i.e., DNA, RNA or
57 proteins) in order to assess which level best correlated with isotope-based measurements of
58 nitrification activity in agricultural soils after fertilization. This work reveals the strengths as well
59 as the associated limitations of metagenomic, metatranscriptomic, and metaproteomic
60 approaches in serving as reliable proxies for examining microbial activities in highly diverse
61 environments like soils.

62

63 INTRODUCTION

64 Even though the central role of microbes in the cycling of nitrogen is recognized, the
65 dynamics and controls of the interrelated microbial nitrogen pathways in agricultural soils are
66 still poorly understood. This scarcity of information limits the development of more accurate,
67 predictive models of nitrogen flux that encompasses the role of microbes in the generation and
68 consumption of nitrogen substrates, as well as the emission of greenhouse gases, including
69 nitrous oxide (N₂O) (1). In agricultural soils receiving large inputs of nitrogen fertilizer, ammonia-
70 oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA) and nitrite-oxidizing bacteria (NOB)
71 collectively are responsible for the conversion of ammonium to nitrate. In addition, the recent
72 discovery of *Nitrospira* bacteria capable of complete oxidation of ammonia to nitrate
73 (comammox) has revealed that the process of nitrification in natural environments might be
74 carried out by a single taxon (2, 3). It has also been reported that nitrification is a major N₂O
75 source under low oxygen concentrations (4), although detailed mechanistic understanding is
76 lacking (5). Alternatively, under anoxic conditions, nitrate (NO₃⁻) can be reduced to gaseous
77 forms such as dinitrogen (N₂), nitric oxide (NO) or N₂O by denitrifying organisms and
78 consequently be lost to the atmosphere. Despite the apparent importance of nitrification in the
79 generation of N₂O and NO₃⁻, the relative contributions of comammox, AOA, AOB and NOB
80 populations in this process, especially during soil fertilization events, is less clear (6). Advancing
81 this issue is essential for better prediction of the contributions of these microbial taxa to the
82 nitrogen cycle and the modeling of the corresponding activities and products. High-throughput
83 sequencing and proteomic approaches offer the means to characterize the nitrogen pathways in
84 the environment. However, to what extent these omic approaches reflect process rates is still
85 unclear.

86 Although DNA, RNA, and protein abundances all reflect microbial potential and
87 responses to environmental changes and thus, can be used to study nitrogen cycling in soils,
88 each measurement generally offers different types of information. For instance, metagenomics

89 (DNA level) offers a comprehensive overview of the functional potential of microbial
90 communities but does not generally reflect active community members or functions. Short-term
91 microbial responses to external changes (e.g., nitrogen addition) can be tracked by analyzing
92 the actively expressed genes (i.e., metatranscriptomics). For instance, the relationship between
93 measured nitrification processes and the ammonia monooxygenase (*amoA*) transcripts have
94 revealed differences between archaeal and bacterial activity in acidic soils (7). Proteomics
95 provides a third level of molecular information much closer to the metabolic processes by
96 reflecting synthesized enzymes that catalyze reactions. Although proteomics has been applied
97 to only a limited number of natural microbial communities, the results have provided new
98 insights about metabolic pathways and interdependencies among microbial groups [reviewed in
99 (8)]. Furthermore, recent advances in metagenomics and metaproteomics techniques as well as
100 integration with isotope-based technologies (e.g., NanoSIMS) have disentangled the role of
101 previously elusive keystone microbial populations. The combined application of metagenomics
102 and metaproteomics has provided new understanding of novel not yet cultured microorganisms
103 participating in the cycling of sulfur, nitrogen, and carbon in the terrestrial subsurface (9).

104 Only a few studies have examined how the above approaches correlate with process
105 rates, especially in soil ecosystems that are characterized by low metabolic activity along with
106 high microbial diversity and heterogeneity. Thus far, almost all studies have provided only
107 qualitative results from applications of omics to soils (10). Quantitative results in a few recent
108 reports have focused mostly on systems with reduced diversity or specific functions and taxa
109 (as opposed to community-wide activities). For instance, metatranscriptomic approaches
110 examining the degradation of the herbicide atrazine by *Escherichia coli* in bioreactors revealed a
111 linear relationship between the measured enzymatic activity and the transcripts encoding the
112 associated enzyme (11). Additionally, in microbial leaf litter decomposition incubations, cellulase
113 and xylanase protein abundances were positively correlated with their corresponding enzymatic
114 activities (12). On the other hand, even though the combination of multi-omic datasets provided

115 new insights into diversity and gene potential of microbial communities of permafrost
116 ecosystems, the datasets were less predictive of measured process rates (13). Therefore, to
117 what extent the omic measurements correlate with each other and with process rates in soils
118 remain unclear.

119 Toward closing this knowledge gap, we examined nitrogen-amended sandy soils
120 obtained from a site with a history of agricultural management and application of synthetic
121 nitrogen fertilizer. A prior year-round characterization of field samples from the same agricultural
122 site revealed increased abundance of novel *Thaumarchaeota* and comammox nitrifiers, but the
123 findings were limited to metagenomics (14). Here, our goal was to assess the strengths and
124 limitations of multi-omics in detecting microbial activity by correlating measurements of DNA,
125 RNA, and protein abundances with measured rates of nitrate formation and N₂O production in
126 soils incubated under controlled conditions in the laboratory. The results reveal that
127 metatranscriptomic data best reflected the measured nitrification rates under the tested
128 experimental conditions.

129

130 **RESULTS**

131 **Nitrification activity in soil microcosms**

132 We first examined nitrification activity in nitrogen-amended microcosms with an
133 equimolar mixture of NH₄⁺ and urea during an eight-day period by following NO₃⁻ formation and
134 NH₄⁺ disappearance. Based on the NH₄⁺ concentration patterns, urea quickly hydrolyzed to
135 release NH₄⁺ within the first two days of incubation (Figure 1a). Specifically, the NH₄⁺
136 concentrations peaked at 48 hours of incubation (18.02 ± 1.5 µgN-NH₄⁺ g⁻¹ dry soil) from urea
137 hydrolysis, and decreased to 5.4 ± 2.5 µgN-NH₄⁺ g⁻¹ dry soil by 192 hours of incubation due to
138 nitrification. Nitrification activity increased five to eight days after the addition of the NH₄⁺ and
139 urea mixture, reaching an average rate of 4.1 ± 0.87 µg N-NO₃⁻ g⁻¹ dry soil d⁻¹ (n = 6) after 192
140 hours of the incubation (Figure 1b). The NO₃⁻ concentrations gradually increased from an initial

141 value of $0.81 \pm 0.28 \mu\text{gN-NO}_3^- \text{ g}^{-1}$ dry soil to 1.91 ± 0.5 at 120 hours of incubation, and then
142 increased at a faster rate to $15.06 \pm 2.7 \mu\text{gN-NO}_3^- \text{ g}^{-1}$ dry soil at 192 hours of incubation (Figure
143 1a). As a result of nitrification activity, pH values decreased across replicated nitrogen-amended
144 microcosms during the incubation (Supplementary Table 1). In order to examine the generation
145 of N_2O possibly generated as a by-product of oxidation reactions during nitrification, we
146 measured the production of N_2O in nitrogen-amended incubations. Net N_2O production rates in
147 the incubation headspace increased from $0.08 \pm 0.006 \text{ ng N-N}_2\text{O g}^{-1}$ dry soil d^{-1} after 24 hours to
148 $0.71 \pm 0.57 \text{ ng N-N}_2\text{O g}^{-1}$ dry soil h^{-1} at the end of the incubations (Figure 1c). Control
149 microcosms receiving only irrigation water (i.e., no nitrogen amendment) did not show net
150 NH_4^+ oxidation.

151 To evaluate possible differences between the use of NH_4^+ or urea in the nitrifying
152 activity, we determined $^{15}\text{NO}_3^-$ production rates using nitrogen stable isotopes in the
153 microcosms. In general, $^{15}\text{NO}_3^-$ production was similar between $^{15}\text{NH}_4^+$ and ^{15}N -urea
154 microcosms, although rates were higher after 10 and 48 hours of incubation (two tailed t -test,
155 $P < 0.01$) in $^{15}\text{NH}_4^+$ and ^{15}N -urea microcosms, respectively, but converged thereafter
156 (Supplementary Figure 1). By the end of the incubations, approximately half of the added ^{15}N
157 was converted to $^{15}\text{N-NO}_3^-$ (49-55% for both labelled solutions/treatments), and only a small
158 fraction converted to $^{15}\text{N-N}_2\text{O}$ (0.006-0.01%). The remaining added nitrogen was presumably
159 converted to N_2 , assimilated into microbial biomass, or adsorbed to soil particles.

160

161 **Soil metagenomes and metatranscriptomes**

162 To explore the genetic potential of microbial communities in control and nitrogen-
163 amended microcosms, we examined the metagenomes and metatranscriptomes obtained from
164 the incubated soils. Metagenomes ranged from 23.7 to 53.4 and metatranscriptomes from 10.1
165 to 31.3 million short-reads per sample (Supplementary Tables 2 and 3). The estimated average
166 coverage based on read redundancy using Nonpareil (15) ranged from 0.27 to 0.42 for the soil

167 metagenomes (values range from 0 to 1). The co-assembly of selected soil metagenomes
168 generated 1.52 million contigs over 500 bp (assembly N50=1,176) and 1.56 million predicted
169 protein-coding genes.

170 A high fraction of ribosomal RNA was detected for all metatranscriptomes ranging from
171 94% to 98% of the total sequences (Supplementary Table 4). No rRNA depletion step was
172 performed during our metatranscriptomic protocol due to overall low total RNA yields from the
173 soils. As expected based on the length of the rRNA genes, 23S rRNA/16S rRNA ratios ranged
174 from 1.7 to 1.9, indicating adequate RNA quality. Bacterial 16S rRNA (16S) was the most
175 abundant, ranging between 30.6% and 35.9% of total transcripts per sample. Archaeal 16S and
176 eukaryotic 18S rRNA molecules were less abundant, with values ranging from 0.09% to 0.15%
177 and 0.55 to 2.9%, respectively.

178

179 **Taxonomy of microbial soil populations based on 16S rRNA gene sequences**

180 The taxonomic composition and abundances of the main microbial groups determined
181 from recovered 16S rRNA (16S) gene sequences (DNA level) from nitrogen-amended
182 incubations, were generally stable during incubations. At the class taxonomic level,
183 *Actinobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria* were the most abundant groups
184 in metagenomes, accounting for more than 57% of the total community in nitrogen-amended
185 incubations (Supplementary Figure 2). The taxonomic composition derived from
186 metatranscriptomes (cDNA reads) was also stable during the incubations but the abundances
187 for main taxonomical groups were substantially different from the metagenomes. For instance,
188 *Betaproteobacteria*, *Gammaproteobacteria*, and *Flavobacteria* were among the most abundant
189 groups in cDNA samples, accounting for an average of 77.5% of the 16S transcripts. In
190 agreement with our previous results based on field samples from the same agricultural site (14),
191 bacterial and archaeal groups associated with the nitrification processes were comparatively
192 less abundant than the aforementioned groups in both DNA and cDNA datasets. For instance,

193 known AOB and NOB genera such as *Nitrosomonas* and *Nitrospira* had average relative
194 abundances of 0.01% and 1.6% of the total populations in the metagenomes from incubated
195 soils. Additionally, the relative abundances of the AOA genera related to *Nitrososphaera* and
196 *Nitrosopumilus* were 0.9% and 0.3% in the microcosm metagenomes. Similar low abundances
197 were determined for known nitrifier genera in metatranscriptomes. Notably, 16S transcript
198 abundances for AOB conspicuously increased during the incubation period (Supplementary
199 Figure 3). In fact, relative 16S gene expression ratios (cDNA/DNA) for AOB and NOB belonging
200 to *Nitrosospira*, *Nitrosomonas* and *Nitrospira* increased 3-, 6-, and 14-fold between 10 and 192
201 hours. In contrast, the 16S gene expression levels for the archaeal groups *Nitrososphaera* and
202 *Nitrosopumilus* were stable during the same incubation period, although with a slight increase in
203 relative expression at 48h of incubation (Supplementary Figure 3).

204

205 **Individual populations from microcosm metagenomes**

206 The assembly and binning of the soil metagenomes recovered 11 metagenome-
207 assembled genomes (MAGs) mostly representing *Proteobacteria*, *Acidobacteria*, *Actinobacteria*
208 and *Nitrospirae* phyla. Most of the recovered MAGs represented novel genera (n=7) and
209 species (n=5) when the taxonomic novelty was evaluated against 10,487 reference genomes
210 (taxonomically classified at the species level) using genome-aggregate amino acid identity (AAI)
211 thresholds for taxonomic rank delineation (16) (Supplementary Table 5). Given that none of the
212 MAGs represented AOA, AOB, NOB, or comammox populations, we included MAGs obtained
213 from a previous analysis of field samples from the same site (Havana county, Illinois, USA) and
214 depth as the soil used in the soil microcosms in the present study (14). MAGs potentially
215 involved in nitrification processes were likely missed in the microcosm metagenomes due to
216 comparatively lower sequencing effort or because of sample heterogeneity (e.g., lower
217 population abundance) but had relatively higher abundance in the previous field samples. The
218 MAGs (designated with the letter F at the end of their name for Field metagenomes) consisted

219 of two complete ammonia oxidizer (comammox) *Nitrospira* MAGs (MAG021F and MAG017F)
220 and five ammonia-oxidizing archaea MAGs representing the *Thaumarchaeota* lineages I.1b
221 (MAG032F and MAG019F) and I.1a (MAG004F, MAG109F, and MAG001F) (Supplementary
222 Figure 4b). The *Nitrospira* MAG007, obtained from the microcosm metagenomes, was closely
223 related to previously described soil comammox (e.g., MAG017F) organisms, sharing 67.8% AAI
224 (SD: 18% based on 2201 shared proteins). However, the MAG007 only encoded a
225 hydroxylamine oxidoreductase (*haoA*) gene and lacked *amoA* and *nxrA* genes (Supplementary
226 Figure 4b). Furthermore, the *Nitrospira* MAG007 formed an independent but related cluster to
227 the soil comammox organisms when reconstructed phylogenies using concatenated single-copy
228 genes were evaluated (Supplementary Figure 5). Thus, AAI values and phylogenetic
229 reconstruction supported the affiliation of MAG007 to *Nitrospira*, but the lack of genes involved
230 in ammonia and nitrite oxidation (possibly due to low sequencing coverage) made it
231 inconclusive whether this taxon is involved in nitrification processes and might indicate
232 divergence from previously described soil comammox organisms.

233 Relative expression values of MAGs (measured as transcripts or reads per kilobase
234 million, RPKM) were used as a proxy for comparing the response and metabolic activity among
235 nitrifying bacteria and archaea during incubations. Even though expression values for most
236 nitrifying MAGs belonging to *Nitrospira* and *Thaumarchaeota* were stable and relatively low,
237 AOA MAGs 004F, 019F and comammox MAG017F, had, on average, the highest expression
238 values throughout the incubations (Supplementary Figure 4a). For instance, the increase in
239 expression values for AOA MAGs belonging to the I.1b clade, 004F and 032F, were 39% and
240 50% after 48 hours of incubation (compared to expression levels at 10 hours incubation),
241 respectively. In contrast, gene expression of comammox MAG017F and *Nitrospira* MAG007
242 increased by 59% and 68% after 120 and 192 hours of incubation, respectively (Supplementary
243 Figure 4a). Note that AOB and NOB were not included in the RPKM analysis due to lack of
244 recovered MAGs representing these populations (see above). Nonetheless, a gene-based

245 approach allowed the analysis of changes in transcript abundances of genes involved in
246 nitrification activity for AOB and NOB nitrifiers (see below).

247

248 **Quantification of nitrification genes in microcosms**

249 To further explore the microbial nitrification processes in incubated soils at the gene
250 level, we specifically quantified gene fragments and transcripts directly involved in nitrification
251 reactions. Relative expression values belonging to the gene encoding urease subunit c (*ureC*)
252 were stable throughout the incubation but average abundances were relatively low compared to
253 other nitrification genes (Figure 2a). The relative expression of the bacterial gene encoding
254 ammonia monooxygenase subunit alpha (*amoA*) was 53.5-fold higher compared to the
255 expression values at 10h of incubation. Most of the detected *amoA* transcripts (cDNA) were
256 phylogenetically affiliated with *Betaproteobacteria* and corresponded to up to 90% of the total
257 detected bacterial *amoA* transcripts at 192 hours of incubation (Figure 2a). Unlike
258 betaproteobacterial *amoA*, abundance of transcripts belonging to comammox were stable
259 throughout the incubation. Furthermore, transcripts belonging to comammox were more
260 abundant compared to *Betaproteobacteria amoA* transcripts after 48 hours of incubation;
261 however, at 192 hours of incubation, betaproteobacterial *amoA* DNA abundance increased 66-
262 fold, whereas comammox *amoA* gene fragments remained stable (Figure 2b). The latter results
263 indicated that the comammox *amoA* may be more abundant under field conditions but
264 betaproteobacterial *amoA* might show a faster response upon ammonia addition, which was
265 also consistent with a previous study (14). Although the relative expression for the archaeal
266 *amoA* was more stable throughout the incubation compared to its betaproteobacterial
267 counterparts, a maximum expression was reached after 120 hours of incubation, suggesting
268 that archaeal *AmoA* activity temporarily increased at later time points during the incubation.
269 Archaeal *amoA* transcripts belonging to the group I.1b were ~7 times more abundant than their
270 I.1a counterpart across the incubations (Figure 2b). Similar to *amoA* patterns, the relative

271 expression for the betaproteobacterial hydroxylamine oxidoreductase (*haoA*; $\text{NH}_2\text{OH} \rightarrow \text{NO}_2^-$)
272 steadily increased during the incubations, whereas comammox *haoA* transcripts represented
273 the remaining smaller transcript fraction and were stable throughout the incubations (Figure 2b).
274 Expression values for the nitrite oxidoreductase subunit alpha (*nxrA*; $\text{NO}_2^- \rightarrow \text{NO}_3^-$) had a 12.4-
275 fold increase compared to the 10-hour time point, consistent with the patterns observed for the
276 previous nitrification genes and NO_3^- accumulation. Unexpectedly, expression values for *nirK*
277 ($\text{NO}_2^- \rightarrow \text{NO}$) affiliated to *Thaumarchaeota* were higher compared to *nirK* transcripts assigned to
278 the *Nitrospira* clade. In fact, *Thaumarchaeota nirK* transcripts had a 3.4-fold increase after 192
279 hours of incubation relative to earlier sampling points, indicating that *Thaumarchaeota* might
280 have been more active in the reduction of nitrite compared to other steps of nitrification.
281 Specifically, there was a 3.4-fold increase for clade I.1b *nirK* transcripts during the 10 to 192
282 hours of incubation period, whereas the abundance of transcripts from clade I.1a were stable
283 throughout the incubations (Figure 2b).

284 In summary, the metatranscriptomic profiles suggested that AOB, but not comammox,
285 responded rapidly to the nitrogen amendment, whereas AOA followed with less pronounced
286 transcriptome shifts. The response of AOB, and to a lesser extent AOA, was also reflected at
287 the DNA level, albeit with a substantial time delay. For instance, shifts were observed early at
288 the transcript level while at the DNA level, changes were mostly evident 192 hours after the start
289 of incubation (Supplementary Figure 6a, b). These results were consistent across the individual
290 nitrification steps and indicated that at least the AOB nitrifiers grew in response to nitrogen
291 addition.

292

293 **A proteomic perspective in soil microcosms**

294 A metaproteomic analysis of the control and nitrogen-amended microcosms at 192
295 hours of incubation detected a total of 2,892 and 1,629 non-redundant peptides, respectively. A
296 total of 844 peptides were shared among control and nitrogen-amended incubations, whereas

297 2,048 and 785 were exclusively present in each microcosm, respectively. Most of peptides
298 detected in control and nitrogen-amended incubations matched protein sequences predicted
299 from metagenomic assemblies (89.4% and 88.2%, respectively) and the remaining fraction
300 matched reference proteomes (Supplementary Table 6). The top 20 most abundant proteins in
301 control and nitrogen-amended treatment microcosms were related to housekeeping and
302 transport proteins whereas in the latter incubation, oxidoreductases for small carbon and alcohol
303 molecules and ATP synthesis were among the most abundant proteins detected
304 (Supplementary Table 7). The taxonomic affiliation, at the class level, for the most abundant
305 annotated peptides belonged to *Alphaproteobacteria*, *Betaproteobacteria*, and *Acidobacteria* in
306 control and nitrogen-amended incubations. Although there were major compositional changes
307 for abundant groups such as *Betaproteobacteria* (40% decrease) and *Gammaproteobacteria*
308 (50% decrease) (Figure 3a), increased abundance was detected for less abundant groups
309 commonly associated with the nitrification process. For instance, close to a 2.2-fold increased
310 abundance for nitrogen-amended incubations were detected for peptides belonging to
311 *Nitrospira*. Detected peptides related to folding and synthesis were the most abundant and had
312 similar abundances in the control and nitrogen-amended microcosms after 192 hours of
313 incubation. However, the relative abundance of ATP synthases and transcription categories
314 were higher in the nitrogen-amended samples relative to the control, presumably as a
315 consequence of a higher microbial activity generated after the nitrogen input. On the other hand,
316 heat-shock and degradation proteins were more abundant in the control incubation, probably
317 reflecting a more prevailing dormant state for the microbial communities in these samples
318 (Figure 3b). However, unlike the metagenomic and metatranscriptomic datasets, only some
319 peptides involved in nitrification were identified using metaproteomics. For instance, the
320 detected peptides directly involved in nitrification pathways corresponded to the nitrite
321 oxidoreductase subunit B (NxrB), which had a 31.3% abundance increase in the nitrogen-
322 amended samples compared to the control.

323

324 **DISCUSSION**

325 **Using multi-omic approaches for examining process rates**

326 Measuring nitrification rates in incubated soils allowed us to evaluate the explanatory
327 and predictive power of omic approaches in a highly diverse soil system. Despite all three omic
328 approaches revealed increased abundance for target genes, transcripts and proteins related to
329 nitrification pathways, they differed in temporal resolution and quantitative capabilities. For
330 instance, the strongest agreement to the observed nitrification processes (i.e., ammonia or
331 nitrite oxidation) was for the metatranscriptomic data within the first days of incubations (e.g.,
332 Figure 2b), whereas metagenomes lagged behind and only reflected the ongoing nitrification
333 process after 192 hours of incubation (e.g., Supplementary Figure 6a, b). These data were
334 presumably attributed to the fact that growth (e.g., at least a few replication cycles) should occur
335 before metagenomics can reveal shifts in relative abundance over time. Note that microbial
336 growth was not explicitly measured by our study to further corroborate the above conclusions
337 and interpretations. Therefore, metagenomics could also reflect underlying microbial processes
338 if the processes are ongoing for a period of time and are coupled with the growth of the
339 corresponding organisms. In contrast, if the goal is to see immediate responses to a
340 perturbation or the perturbation is short-lived (e.g., lasting a few hours), metatranscriptomic data
341 will be preferable. We also observed that metatranscriptomes were as good as metagenomics,
342 if not better, at reflecting microbial activity for nitrification processes even at later incubation time
343 points. In contrast, the metaproteomes offered, at most, a qualitative glimpse at nitrification
344 processes and were less definitive in identifying common nitrification markers. The latter was
345 largely attributable to the computational challenges associated with proteomic data such as high
346 peptide redundancy and the requirement of high-quality assemblies which are still challenging
347 for highly complex soil metagenomes. Furthermore, many challenges remain for efficient

348 extraction of membrane proteins from low abundance organisms such as nitrifiers. Ultimately,
349 these technical limitations could be reflected in a lower number of detected proteins compared
350 to the number of metagenomic and metatranscriptomic reads recovered that encoded the
351 proteins of interest.

352 While shifts in 16S gene ratios (cDNA/DNA) were relatively small for AOA, the 16S and
353 functional gene ratio shifts (e.g., *amoA*) for AOB/NOB were much more pronounced throughout
354 the incubations (Supplementary Figure 3). Nonetheless, there were changes in transcript
355 abundances for nitrification genes from both microbial groups in the microcosms (Figure 2).
356 These results might reflect an active and growing state for AOB/NOB and mostly active AOA
357 communities as observed before for agricultural soil microcosms (17). The differences observed
358 between target gene abundances and 16S gene ratios from AOA could reflect a limitation of the
359 latter data when used as a proxy for assessing microbial activity (18). However, more frequent
360 sampling and incubations under different physicochemical conditions will be required for more
361 robust conclusions to emerge on the exact relationship(s) between molecular level information
362 and process rates. The results reported here provided an overview of this relationship for soils
363 and are highly promising for the future.

364 In terms of the ecological adaptation of the nitrifiers analyzed here, the Havana
365 agricultural site has had a long history of cyclical seasonal inputs (e.g., fertilizers) that have
366 shaped the structure of microbial communities in different soil layers. The AOA and AOB
367 communities in the Havana site have legacy establishments at the 20-30 cm soil depth and are
368 under relatively stable environmental conditions compared to the top soil layer (14). Thus,
369 nitrogen amendments tested in our experiment and experimental conditions might not represent
370 closely the conditions usually experienced by the examined AOA and AOB communities. The
371 rapid response of AOB observed here might be a reflection of physiological adaptations of AOB
372 to thrive under high nitrogen content as reported previously (17). In contrast, the low response
373 observed for comammox and some AOA communities might reflect their limited physiological

374 capabilities to respond to high nitrogen concentrations (2, 3) that were assayed in our
375 experimental setup.

376 Previous authors have also found metatranscriptomic approaches to be better predictors
377 of measured microbial activity (11) in controlled laboratory systems amended with exogenous
378 organic compounds, but have been more limited in providing insights into the whole-microbial
379 community response to the amendment. For instance, the changes in transcripts observed at
380 early incubation points for specific lineages (e.g., comammox vs. betaproteobacterial *amoA*)
381 suggested ongoing microbial activity that became evident only at the DNA level (relative
382 abundance) at the last incubation point in the metagenomes (Figure 2b). Future incubation
383 studies could shed light on the intrinsic differences between nitrifier (and denitrifier) communities
384 by testing variables such as oxygen availability (i.e., water saturation) and different agricultural
385 soil types. For instance, the incubation conditions used in our study deliberately promoted
386 nitrification over denitrification processes and as a result, the N₂O production was detected due
387 to the former process. Consequently, nitric oxide (e.g., *norB*) and nitrous oxide reductases (e.g.,
388 *nosZ*) transcripts, which are responsible for N₂O production and consumption during
389 denitrification, respectively, were not detected in our metatranscriptomes datasets (i.e.,
390 abundance below detection limit). Also, the use of nitrification inhibitors could help to elucidate
391 the origin of the measured N₂O whether production was biotic or abiotic, for which our data are
392 limited in predicting. Thus, the integration of *in situ* rates along with the microbial dynamics
393 examined by metatranscriptomes and metagenomes could provide the means to better
394 understand and predict nitrification and N₂O emission in agricultural soils.

395 **New insights into nitrification pathways**

396 The metagenomic and metatranscriptomic datasets combined with phylogenetic
397 approaches provided a closer examination of the poorly studied microbial diversity in agricultural
398 soils. Assessing the individual gene level, as opposed to whole genome transcript level,

399 provided more robust results for relating population response to measured nitrification reactions,
400 presumably due to higher sequence coverage (less noise). Even though a direct comparison at
401 the genome level between AOB, NOB and comammox AOB was not possible due to the lack of
402 recovered MAGs representing AOB and NOB populations, analysis of individual 16S and
403 nitrogen cycling genes elucidated the importance of AOB and NOB in the microcosm
404 experiments. Our results showed that even though betaproteobacterial *amoA* transcripts
405 responded to the addition of ammonium and urea, the relative abundance of comammox *amoA*
406 transcripts was stable (i.e., not responding to the nitrogen amendment), although comammox
407 populations were relatively more abundant than AOB in the microcosms. This observation is
408 consistent with previous metagenomic results from the same agricultural soil, where comammox
409 *amoA* genes and the organisms encoding these genes represented the highest fraction of
410 nitrifying bacteria (14). The differences between measured genes and transcripts indicated that
411 the incubation conditions favored the activity of *Betaproteobacteria* over comammox nitrifying
412 bacteria, suggesting ecophysiological differences among these taxa for the incubation
413 conditions or added substrates compared to field conditions.

414 The sequencing of isolates and environmental AOA genomes has shown that even
415 though they encode an AmoA protein, they lack a canonical hydroxylamine oxidation pathway
416 (19). Previous studies have proposed that nitric oxide is essential for hydroxylamine oxidation to
417 nitrite in archaea (20). The proposed mechanism involves oxidation of ammonium to
418 hydroxylamine followed by oxidation to nitrite catalyzed by a putative Cu-protein that uses nitric
419 oxide as co-reactant for the oxidation of hydroxylamine. Interestingly, nitric oxide has been
420 proposed to be derived from the activity of the NirK enzyme present in all AOA sequenced
421 genomes. Our results show that unlike AOA *amoA* or bacterial *nirK* transcripts,
422 *Thaumarchaeota nirK* transcripts increased in abundance in the incubated soils, supporting the
423 abovementioned hypothesis. Therefore, even though AOA *amoA* transcripts did not show clear
424 changes in abundances compared to their bacterial counterparts, these results might be in

425 agreement with the previous hypothesis, and likely denote an unaccounted role for
426 *Thaumarchaeota nirK* in nitrification in agricultural soils.

427 **Multi-omic limitations**

428 Soil samples are challenging to analyze not only because of their heterogeneous
429 structure and chemical composition, but also because of the highly diverse microbial
430 communities and slow growth kinetics. Despite the advancements presented here, there are still
431 opportunities for further improvements. For instance, here we analyzed total RNA extractions
432 from soils where ribosomal rRNA transcripts represented 94-98% of the total sample, limiting
433 our study to a small fraction of transcripts related to functional genes. Current experimental
434 approaches offer successful rRNA depletion for environmental samples, when RNA yields are
435 not limiting (21). Additionally, all the results represented here provide only relative abundances
436 for measured microbial markers. For instance, approaches such as qPCR or internal standards
437 spiked into the DNA or cDNA library for sequencing (21) can strengthen and provide improved
438 quantification compared to those presented here.

439 Metaproteomics offered an additional layer of information for the microbial activity, but it
440 was less comprehensive compared to metagenomes and metatranscriptomes. Even though our
441 database for proteomic analyses included a high fraction of nitrification proteins predicted from
442 these agricultural soils, only peptides belonging to the NxrB were detected. The results obtained
443 were attributable, at least partially, to the low biomass, especially for the low abundance
444 nitrifiers targeted here. Further, possible protein extraction biases due to the complexity of soil
445 matrices as well as limited extraction of membrane proteins, such AmoA, might have also
446 influenced the outcome of our efforts (22). Nonetheless, the abundances for several peptides
447 belonging to housekeeping proteins of nitrifier organisms were increased during the incubation
448 time, consistent with the results from metagenomic and metatranscriptomic approaches.
449 Therefore, metaproteomics provided a qualitative confirmation of the underlying nitrification

450 processes ongoing during our incubations and of the responsible taxa. Alternative proteomic
451 approaches focused on a preselected set of proteins (i.e., selected reaction monitoring or target
452 proteomics) could be used to explore low abundance nitrification proteins. For instance,
453 targeted proteomic approaches have been used to study proteins in low abundance involved in
454 bioremediation pathways in highly-diverse environmental systems (23). Therefore, targeted
455 proteomics might offer new opportunities for researchers interested in detecting low-abundance
456 peptides and prediction of process rates in complex samples (24).

457 The analyses of different omic levels obtained from the incubations showed a high
458 correspondence between nitrification gene markers and nitrification process rates. The gene
459 fragments and transcripts were mostly affiliated to novel nitrifier populations similar to those
460 previously described in field soil metagenomes from the same agricultural site (14). Therefore,
461 the gene and genome sequences reported here could facilitate future investigations of nitrogen
462 cycling in agricultural fields; for instance, by applying qPCR assay targeting the key taxa and
463 biomarker genes and transcripts. The combination of metagenomic and metatranscriptomic
464 approaches used in our study provided a promising strategy for examining microbial activity in
465 agricultural soil environments. Therefore, the findings presented here highlighted the potential of
466 omics data to serve as reliable proxies for examining microbial processes *in situ*, especially in
467 soils, which has been proven to be among the most challenging tasks for environmental studies.

468

469 **MATERIALS AND METHODS**

470 **Soil Sampling**

471 Our study was focused on an agricultural plot located in the Havana County, Illinois,
472 USA (lat 40.296, long 89.944; elevation, 150 m). The site is representative of the US Midwest
473 and has a long history of conventionally managed corn and soybean crop rotation. In October
474 2014, we collected ~2 kg of bulk soil from a 20-30 cm soil depth as previous results have shown
475 significant presence of ammonia-oxidizing microorganisms in this layer (14).

476

477 **Soil Incubations, Gas and Chemical Analyses**

478 Soil microcosms were established in triplicates, using ~120 g of soil (~8% moisture
479 content) in 500 ml gas-tight canning jars equipped with gas sampling ports, and were sampled
480 at six time points (0, 10, 24, 48, 120, and 192 hours). To set up the microcosms, 6 ml of 40 mM
481 NH_4Cl and 20 mM urea (80 mM N) in water used for irrigation at the site was added to two
482 separate batches of 400 g of soil (Final concentration= 1.2 $\mu\text{moles-N/g}$ or 18.3 $\mu\text{g-N/g}$ dry
483 weight). Two stable isotope treatments were done, one for NH_4Cl (50% $^{15}\text{N-NH}_4\text{Cl}$ and 50% $^{14}\text{N-}$
484 NH_4Cl) and one for urea (50% $^{15}\text{N-NH}_2\text{CONH}_2$ and 50% $^{14}\text{N- NH}_2\text{CONH}_2$). The two treatments
485 allowed for differentiating how the products of nitrification differed between urea and NH_4Cl
486 when both were present. After vigorously mixing, 120 g were dispensed into three separate
487 microcosm jars and incubated in a dark growth chamber with diurnal temperature fluctuation of
488 22-24 °C as observed in Havana field soil at 20-30 cm during the spring fertilization period (early
489 June). Triplicate microcosms each receiving 6 ml of filtered irrigation water (no nitrogen
490 amendment) served as controls. After each sampling point, headspace gas was collected from
491 closed jars and the N_2O concentration was measured on a Shimadzu GC-2014 gas
492 chromatograph (Columbia, MD) equipped with an electron capture detector. Jars were opened
493 for soil sampling and to reestablish equilibration with atmospheric air before being resealed until
494 the next sampling. Residual ammonium and nitrate in soil subsamples (20 g) were extracted in
495 2 M KCL and the concentrations were determined using colorimetric analysis on a flow injection
496 auto-analyzer (Lachat Instruments, Milwaukee, WI) (25). Soil pH (1:1 in water) and gravimetric
497 water content were measured at each time point (Supplementary Table 1). ^{15}N isotopic
498 composition of N_2O in collected jar headspace samples was determined using an IsoPrime 100
499 isotope ratio mass spectrometer interfaced with an IsoPrime trace gas analyzer (Cheadle
500 Hulme, UK) at the University of Illinois at Urbana-Champaign. The ^{15}N atom % enrichment of
501 the NO_3^- pool was determined using acid trap diffusion (26) and analysis of the diffusion disks

502 on a Vario Micro Cube elemental analyzer (Elementar, Hanau, Germany) interfaced to an
503 IsoPrime 100 continuous flow isotope ratio mass spectrometer (Cheadle Hulme, UK). $^{15}\text{NO}_3^-$
504 and $^{15}\text{N}_2\text{O}$ production rates were calculated from the change in $^{15}\text{NO}_3^-$ and $^{15}\text{N}_2\text{O}$
505 concentrations, respectively, from one time point to the following sampling time point. NO_3^- and
506 N_2O production rates were estimated from the $^{15}\text{NO}_3^-$ and $^{15}\text{N}_2\text{O}$ production rates based on the
507 mean ^{15}N excess atom % of the NH_4^+ source pool (27). No inhibitors of nitrogen cycle pathways
508 were used in the incubations.

509

510 **Nucleic Acid Extractions**

511 DNA was extracted from ~0.5 g of soil using a modified phenol-chloroform and
512 purification protocol as previously described (28). For RNA extraction, 2 gr of soil was preserved
513 in LifeGuard (MoBio) and stored at -80°C . A modified protocol derived from the PowerMax Soil
514 DNA kit for extracting RNA was used for total RNA extractions (MoBio). TURBO DNase
515 (Ambion) was used to remove DNA according to the recommendations of the manufacturer.
516 Nucleic acid extracts were quantified using Quant-it ds DNA HS and HS RNA assays
517 (Invitrogen) according to the instructions of the manufacturer. RNA quality was assessed using
518 Agilent RNA 6000 pico kit (Agilent Technologies) and samples having RNA integrity number
519 (RIN) above 7 were used.

520

521 **Nucleic Acid Sequencing**

522 For metagenomes, dual-indexed DNA sequencing libraries were prepared using the
523 Illumina Nextera XT DNA library prep kit according to manufacturer's instructions, except that
524 the protocol was terminated after isolation of cleaned amplified double stranded libraries. For
525 metatranscriptomes, single-indexed cDNA sequencing libraries were prepared using ScriptSeq
526 v2 protocol using ~25 ng of total RNA as input. All DNA and cDNA library concentrations were

527 determined by fluorescent quantification using a Qubit HS DNA kit and Qubit 2.0 fluorometer
528 (ThermoFisher Scientific) according to manufacturer's instructions and samples were run on a
529 High Sensitivity DNA chip using the Bioanalyzer 2100 instrument (Agilent) to determine quality
530 and average library insert sizes. An equimolar mixture of the libraries was sequenced on an
531 Illumina HiSEQ 2500 instrument (School of Biological Sciences, Georgia Institute of
532 Technology) for a rapid run of 300 cycles (2 x 150 bp paired end) using the HiSeq Rapid PE
533 Cluster Kit v2 and HiSeq Rapid SBS Kit v2 (Illumina). Adapter trimming and demultiplexing of
534 sequenced samples was carried out by the Illumina software, according to the
535 recommendations of the manufacturer.

536

537 **Short-read Analyses**

538 Metagenomic and metatranscriptomic raw reads (FASTQ) for all samples were trimmed
539 using SolexaQA (29) using a Phred score cutoff of 20 and minimum fragment length of 50 bp.
540 Short-reads derived from metatranscriptomes were merged using PEAR using default
541 parameters (30). Average coverage for each sequenced metagenome was determined by
542 Nonpareil (15) using default settings except that 2,000 reads were used as query (-X option)
543 (Supplementary Tables 3 and 4).

544 Short-read sequences encoding 16S rRNA gene fragments were extracted from each
545 metagenome and metatranscriptome by SortMeRNA (31) and their taxonomy was assigned
546 using RDP classifier (cutoff 50) (32).

547 To identify and quantify reads encoding specific protein sequences of interest, we used
548 the previously published protein sequences as references (14) for the archaeal ammonia
549 monooxygenase alpha subunit (AmoA), bacterial AmoA, hydroxylamine oxidase (HaoA), nitrite
550 oxidoreductase alpha subunit (NxrA), nitrite reductase (NirK), nitric oxide reductase beta subunit
551 (NorB), nitrous oxide (NosZ), nitrite reductase (NrfA) and DNA-directed RNA polymerase
552 subunit beta (RpoB). Independent ROCKER (33) models (length=125 bp) were subsequently built

553 based on these reference protein sequences with the exception of NarG and NxrA, where the
554 sequences were combined into a single model. Trimmed short-reads from soil metagenomes
555 were used as query for BLASTx searches (e-value 0.01) against the latter protein databases
556 and outputs were filtered using the previously generated ROCKER models. For metagenomes,
557 target gene abundance in metagenomes was determined as genome equivalents by calculating
558 the ratio between normalized target reads (number of reads matching divided by median protein
559 length) and normalized RpoB reads (number of reads matching divided by median RpoB protein
560 length), a universal single-copy gene. For metatranscriptomes, target transcripts abundance
561 was calculated as reads per kilobase of transcript per million mapped reads (RPKM). Protein
562 databases and ROCKER models are available through <http://enve-omics.ce.gatech.edu/>.

563

564 **Assembly and Binning of Metagenomic Populations**

565 Short-read metagenomes from control and treatments (t=0,120 and 192 hours) were co-
566 assembled using IDBA_UD v1.1.1 (34) and binning was performed as previously described
567 (14). Taxonomic classification and degree of novelty (novel species, genus, etc) of the MAGs
568 were obtained from the Microbial Genomes Atlas (MiGA) webserver (35). MAG abundance was
569 determined as the total length of all matching metagenomic or metatranscriptomic reads to the
570 binned contigs from BLASTn searches (identity $\geq 98\%$ and fraction of read aligned $\geq 50\%$)
571 divided by the metagenomic or metatranscriptomic sample sizes (in millions of reads) and the
572 length of the bin genomes in Kbp (Kilo base pairs). Reads encoding rRNA sequences (such as
573 5S, 5.8S, 16S, and 23S) were identified by SortMeRNA, and removed for non-rRNA analyses in
574 order to avoid overestimating abundances.

575 Phylogenetic reconstruction of MAGs was performed based on the concatenated
576 alignment of universal single-copy proteins identified for each bin using the “HMM.essential.rb”
577 script of the enveomics collection (36). For this, thirty bacterial proteins present in the
578 corresponding bins MAGs were extracted and multiple alignments for each protein were

579 generated using ClustalΩ. Concatenated alignments without invariable sites were generated for
580 archaeal and bacterial alignments using the script “Aln.cat.rb”. Phylogenetic reconstructions
581 were determined using in RAXML v8.0.19 (-f a, -m PROTGAMMAAUTO, -N 100) and visualized
582 in iTol.

583 N cycle protein sequences in the co-assembly and MAGs were detected using hidden
584 Markov models obtained from FUNGENE (37), using HMMer (38). Detected target N cycle
585 proteins were manually curated, when necessary, by assessing the presence of characteristic
586 amino acid and phylogenetic congruency.

587

588 **Phylogenetic Trees and Placement of Short-reads**

589 To assess the phylogenetic affiliation of metagenomic or metatranscriptomic reads, reference
590 and fully assembled protein sequences were aligned using ClustalΩ (39) with default
591 parameters. Resulting alignments were used to build phylogenetic trees in RAXML v8.0.19 (40).
592 Short-reads encoding the protein of interest were extracted from metagenomes or
593 metatranscriptomes using ROCKER (BLASTx) and placed in their corresponding phylogenetic
594 tree using the methodology previously described (14). Quantification of the number of reads
595 assigned to a specific clade (e.g., to distinguish between *nxrA* or *narG* reads) was done using
596 the “JPlace.distances.rb” script, also available in the enveomics collection. To quantify *nirK*
597 gene fragments assigned to specific clades, the same process as described above was
598 repeated except that all reads detected by multiple ROCKER models to previously described
599 clades (41) (clades I+II, III and *Thaumarchaeota*) were used.

600

601 **Shotgun Metaproteomics**

602 Approximately 10 g of soil were collected from the 192 hours control and ¹⁵N-
603 NH₄⁺ amended microcosms and stored at -80°C. Frozen soil (5 g) was thawed and suspended
604 in lysis buffer and boiled for 15 minutes as described previously (42). The supernatant was

605 retained and amended with 100% chilled TCA to final concentration of 25% (vol/vol) and kept at
606 -20°C overnight. Samples were centrifuged at 21,000 x *g* for 20 min and the protein pellets
607 processed as described previously (43) and solubilized in 6 M guanidine buffer (6 M guanidine;
608 10 mM dithiothreitol [DTT] in Tris-CaCl₂ buffer (10 mM Tris; , pH 7.8) with 3 hr incubation at
609 60°C. An aliquot of 25 µl/ sample was retained for protein estimation and the rest of the protein
610 sample was digested, peptides desalted and solvent exchanged as described earlier (44). The
611 amount of protein extracted from each sample was calculated using the RC/DC protein
612 estimation kit (Bio-Rad Laboratories, Hercules, CA, USA) as per the manufacturer's instructions.
613 Bovine serum albumin (supplied with the kit) was used as standard for the assay.

614 All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO), unless specified
615 otherwise. High performance liquid chromatography- (HPLC-) grade water and other solvents
616 were obtained from Burdick & Jackson (Muskegon, MI), 99% formic acid was purchased from
617 EM Science (Darmstadt, Germany) and sequencing-grade trypsin was acquired from Promega
618 (Madison, WI).

619

620 **NanoLC-MS/MS Analysis.**

621 Peptides (75 µg) were loaded onto in-house prepared biphasic resin packed column
622 [SCX (Luna, Phenomenex, Torrance, CA) and C18 (Aqua, Phenomenex, Torrance, CA)] as
623 described earlier (44, 45) and subjected to an offline wash for 15 min as previously described
624 (46). The sample column was aligned with an in-house C18 packed nanospray tip (New
625 Objective, Woburn, MA) connected to a Proxeon (Odense, Denmark) nanospray source as
626 previously detailed (46). Peptides were eluted and subjected to chromatographic separation and
627 measurements via 24-hr Multi-Dimensional Protein Identification Technology (MuDPIT)
628 approach as described earlier (44-46). Measurements were carried out using LTQ mass
629 spectrometer (Thermo Fisher Scientific, Germany) coupled to the Ultimate 3000 HPLC system

630 (Dionex, USA) and operated in data dependent mode, via Thermo Xcalibur software V2.1.0 as
631 described earlier (45).

632 For protein identification, the raw spectra from each run were searched against a custom
633 database and was constructed using protein sequences predicted from metagenome
634 assemblies obtained from the same soil and 20-30 cm depth (14), metagenome assemblies
635 from incubations (Supplementary Table 2), and reference proteomes for 47 common soil
636 organisms (Supplementary Table 5). These predicted proteins were used for constructing a
637 database for metaproteomic searches (available through [http://](http://enve-omics.ce.gatech.edu/data/multiomics-soil) [http://enve-](http://enve-omics.ce.gatech.edu/data/multiomics-soil)
638 [omics.ce.gatech.edu/data/multiomics-soil](http://enve-omics.ce.gatech.edu/data/multiomics-soil)). Database matching was done via Myrimatch v2.1
639 algorithm (47) set to parameters described before (48) with minor modifications where static
640 cysteine and dynamic oxidation modifications were not considered. Identification of at least two
641 peptides per protein (one unique and one non-unique) sequence was a prerequisite for protein
642 identifications. Common contaminant peptide sequences from trypsin and keratin were
643 concatenated to the database. Reverse database sequences were also included in the
644 database as decoy sequences to calculate false discovery rate (FDR). For data analysis,
645 spectral counts of identified peptides was normalized as described before (49) to obtain the
646 normalized spectral abundance factor (NSAF) and the NSAF values were multiplied by a
647 constant number (100,000) for better visualization and referred to as normalized spectral counts
648 (nSpc). The nSpc were used to compare expression of proteins across different samples and
649 different time points. Detected proteins predicted from metagenomic assemblies were annotated
650 using BLASTp (50) and UniProt database as reference (51) (downloaded in May of 2017).

651

652 **Accession numbers**

653 Raw metagenomic and metatranscriptomic soil datasets and MAGs are deposited in the
654 European Nucleotide archive under study number PRJEB27434.

655

656

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663

664 **Figure Legends**

665

666 **Figure 1. Nitrogen pools and fluxes in soil incubations amended with NH₄⁺ and urea.**

667 Mean NH₄⁺ and NO₃⁻ concentrations (A), total NO₃⁻ production rate (B), and total N₂O production
668 rate (C) for the nitrogen-amended and control (irrigation water only) microcosms at each
669 incubation time point. Error bars represent the standard deviation from replicate samples (n=6
670 for nitrogen-amended and n=3 for control).

671

672 **Figure 2. Nitrification genes in incubated soils.** A. Relative expression ratios for each

673 nitrification step in incubated soils were determined at 10, 48, 120, and 192 hours incubation. B
674 and C show determined RPKM values for bacterial *amoA* (B), *hao* (C), thaumarchaeotal *amoA*
675 (D), and *nirK* (E) transcripts from metatranscriptomes.

676

677 **Figure 3. Metaproteomic analyses of incubated soils at 192 hours of incubation.** Panel A

678 shows taxonomic affiliation (class) and abundance (average spectral counts) for peptides
679 detected in control and N-amended incubations. Panel B shows summarized functional
680 annotation of detected peptides using SEED functional categories.

681

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683

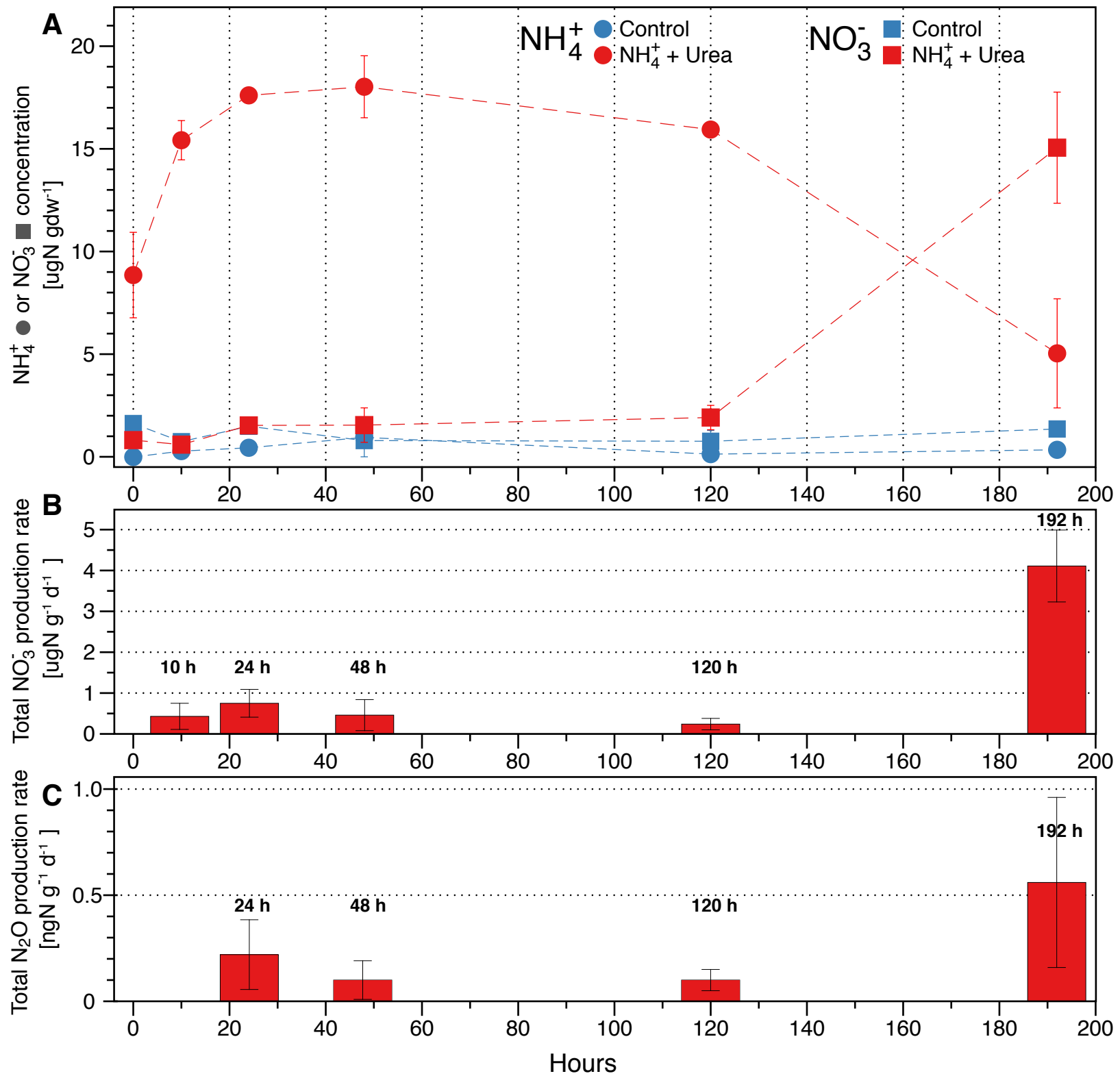
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Figure 1

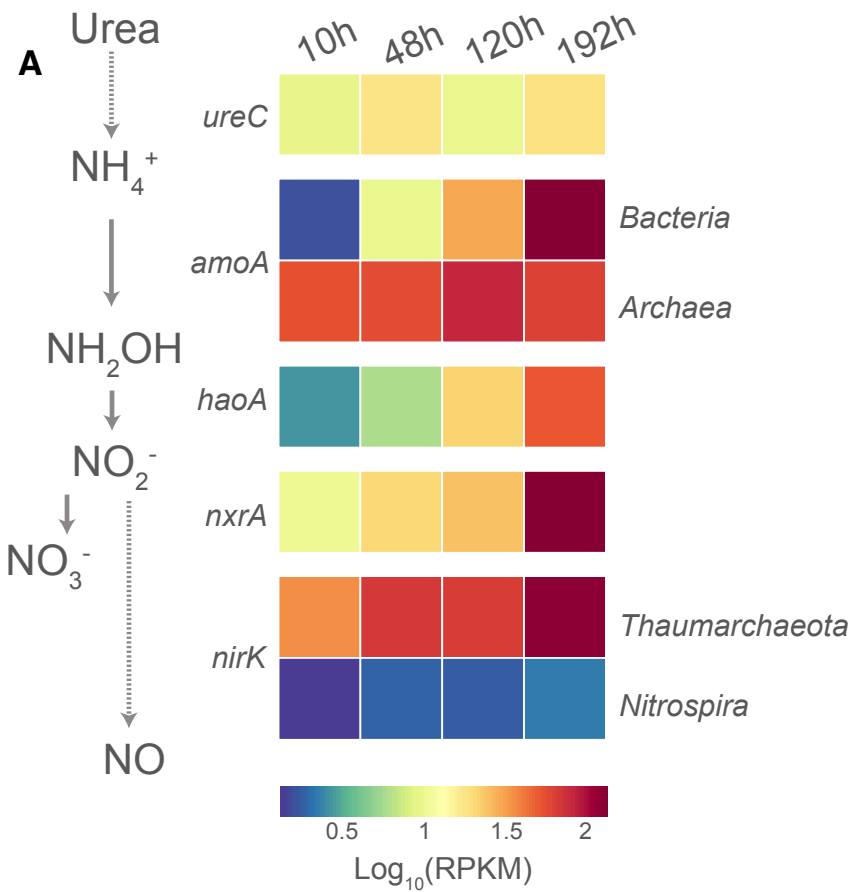


Figure 2

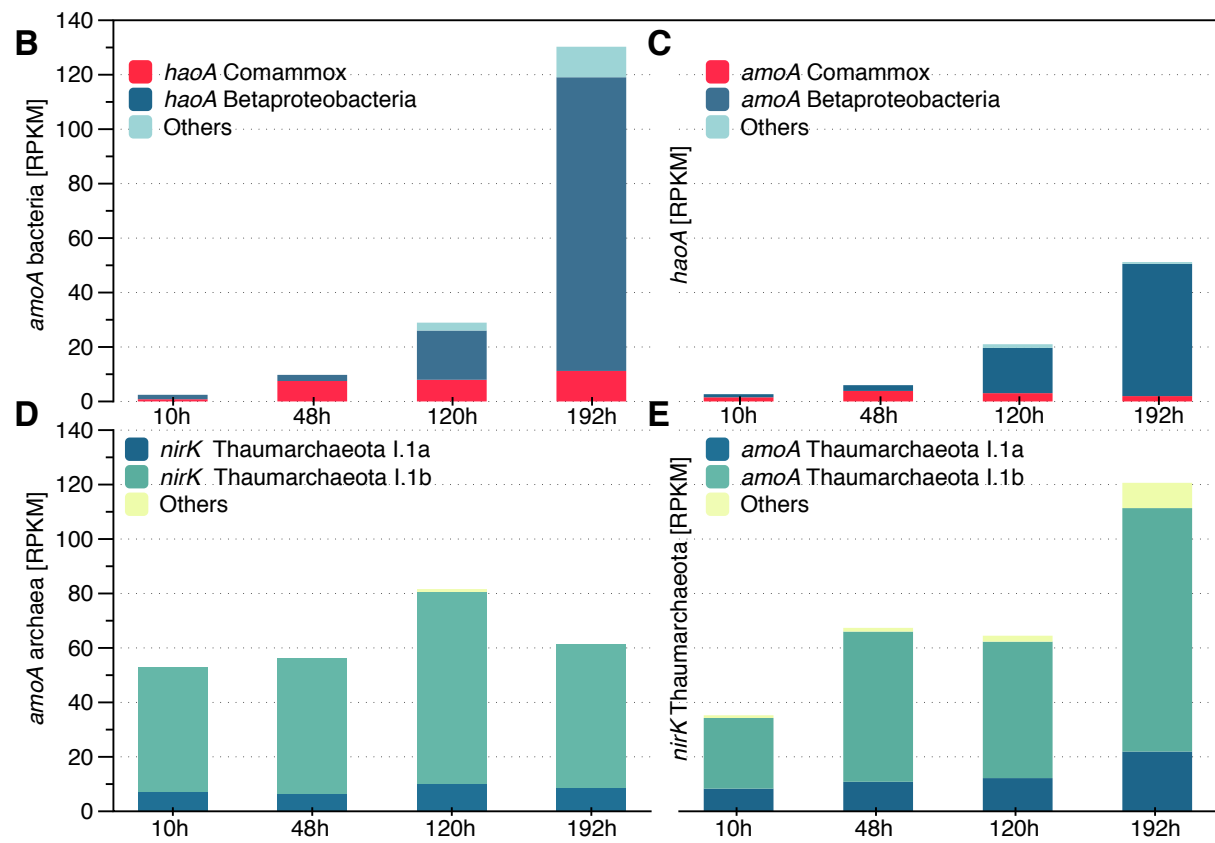


Figure 3

