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TITLE: Rapid response of nitrogen cycling gene transcription to labile carbon amendments in a soil microbial community

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ABSTRACT:

Episodic inputs of labile carbon (C) to soil can rapidly stimulate nitrogen (N) immobilization by soil microorganisms. However, the transcriptional patterns that underlie this process remain unclear. In order to better understand the regulation of N cycling in soil microbial communities, we conducted a 48 h laboratory incubation with an agricultural soil where we stimulated the uptake of inorganic N by amending the soil with glucose. We analyzed the metagenome and metatranscriptome of the microbial communities at four timepoints that corresponded with changes in N availability. The relative abundances of genes remained largely unchanged throughout the incubation. In contrast, glucose addition rapidly increased transcription of genes encoding for ammonium and nitrate transporters, enzymes responsible for N assimilation into biomass, and genes associated with the N regulatory network. This upregulation coincided with an increase in transcripts associated with glucose breakdown and oxoglutarate production, demonstrating a connection between C and N metabolism. When concentrations of ammonium were low, we observed a transient upregulation of genes associated with the nitrogen fixing enzyme nitrogenase. Transcripts for nitrification and denitrification were downregulated throughout the incubation, suggesting that dissimilatory transformations of N may be suppressed in response to labile C inputs in these soils. These results demonstrate that soil microbial communities can respond rapidly to changes in C availability by drastically altering the transcription of N cycling genes.

IMPORTANCE:

A large portion of activity in soil microbial communities occurs in short time frames in response to an increase in C availability, affecting the biogeochemical cycling of nitrogen. These changes

39 are of particular importance as nitrogen represents both a limiting nutrient for terrestrial plants as
40 well as a potential pollutant. However, we lack a full understanding of the short-term effects of
41 labile carbon inputs on the metabolism of microbes living in soil. Here, we found that soil
42 microbial communities responded to labile carbon addition by rapidly transcribing genes
43 encoding proteins and enzymes responsible for inorganic nitrogen acquisition, including nitrogen
44 fixation. This work demonstrates that soil microbial communities respond within hours to carbon
45 inputs through altered gene expression. These insights are essential for improved understanding
46 of the microbial processes governing soil organic matter production, decomposition, and nutrient
47 cycling in natural and agricultural ecosystems.
48

INTRODUCTION

Inorganic nitrogen (N) availability in soil dictates several ecosystem-level processes such as plant growth (1), greenhouse gas emissions in the form of nitrous oxide (2), and eutrophication from runoff (3). The transformation of N by soil microbial communities is directly tied to the pool of bioavailable N in soils (4, 5). Thus, understanding the controls of N metabolism in soil microbes is key to determining, and potentially managing (6), the cycling of N in soils. Although genes and regulatory mechanisms for microbial N cycling processes have long-been identified in laboratory studies (7–9), the short-term dynamics and controls of N cycling in complex soil communities remain poorly understood. The availability of shotgun sequencing technologies to analyze microbial functioning in soil communities provides an opportunity to enhance our understanding of microbially mediated soil N cycling.

Measuring short-term responses of soil microbial populations to changes in the environment is crucial in understanding the role of microbes in biogeochemical cycling. Most biogeochemical transformations occur during short periods of intense microbial activity, when the active fraction of microbes may be up to 20 times higher than in bulk soil (10). This stimulation is often the result of a localized increase in nutrient concentrations, such as in the rhizosphere or an area of fresh organic matter decomposition. Despite the importance of these "hot moments", only a few studies (e.g. 11, 12) have tracked changes in N-cycling gene transcription in soils.

Notably, the short-term (hours to days) transcriptional response of N-cycling genes in response to labile C inputs has yet to be determined. Microbial communities experience sudden changes in C and N availability associated with plant root exudation (13), trophic interactions (14, 15), and litter leachate (16). Since soil microbes are typically limited by labile C and energy

(17–19), the addition of a C-rich substrate is expected to stimulate growth and activity (20), increasing the demand for N (21). Whether N is derived from the uptake of organic N present in the substrate or mineral N available in the soil depends largely on the C:N of the substrate (22). For example, in Yang et al. 2016 (23) soil microbial communities assimilated organic N during the mineralization of added glycine, but in the presence of glucose the mineralization of glycine was initially suppressed and ammonium served as the main source of N. Simple sugars such as glucose have accordingly been shown to influence protease activity (24). The metabolic pathways for N immobilization have been well characterized *in vitro* (25). A majority of N assimilation into biomass occurs through the conversion of NH_4^+ into the amino acids glutamine and glutamate, which are used as sources of N for all other amino acids. Under low-to-moderate intracellular concentrations of NH_4^+, the enzymes glutamine synthetase (GS; encoded by *glnA*) and glutamate synthase (GOGAT; *gltS*) convert NH_4^+ to glutamate in a two-step reaction referred to as the GS-GOGAT pathway (26). Under high concentrations of NH_4^+, the enzyme glutamate dehydrogenase (GDH; *gudB, gdhA*) converts NH_4^+ directly to glutamate in a one-step reversible reaction (27).

Since both the GS-GOGAT pathway and GDH require N as NH_4^+, other forms of inorganic N must be converted to ammonium before conversion into biomass. In the case of nitrate and nitrite, the reduction to ammonium occurs through either assimilatory nitrate reduction or, under anoxic conditions, dissimilatory nitrate reduction to ammonium (DNRA; Table S1) (28). The conversion of atmospheric N_2 to ammonium by diazotrophs is catalyzed by the enzyme nitrogenase (*nifDHK*) (29).

The mechanisms regulating N uptake in response to C have been extensively studied *in vitro* (8, 25). The complex regulatory network includes a specialized sigma factor (σ^{54}; *rpoN*),

three transcriptional regulators, and a phosphorylation cascade comprised of post-modification enzymes, PII proteins, and a two-component regulator (30). The activity of many of the enzymes and proteins in the phosphorylation cascade is tightly controlled by cellular concentrations of glutamine and oxoglutarate (31). Since the concentration of oxoglutarate is impacted by the activity of the TCA cycle, the regulation of N cycling is directly tied to C metabolism (32).

Carbon substrate addition is also thought to influence dissimilatory N cycling processes such as nitrification and denitrification. In nitrification, ammonia is oxidized to nitrite and then nitrate. Often the steps of this process occur in different organisms (33), however complete ammonia oxidizers have also been described (34, 35).". In denitrification, nitrate is reduced to nitrite, nitric oxide, and then nitrous oxide and N_2. Nitrification and denitrification, beyond their ability to draw from the pools of ammonium and nitrate, also represent important avenues of inorganic N loss from soils via nitrate leaching and the release of N_2 and nitrous oxide, a potent greenhouse gas (36). The addition of glucose is expected to have both positive and negative effects on nitrification. Rates of autotrophic nitrification tend to decrease as heterotrophs outcompete autotrophic nitrifiers for ammonium (37), but rates of heterotrophic nitrification may increase after labile C inputs (38). Denitrification is more directly influenced by C availability and quality (39), and the abundance of mRNA transcripts associated with denitrification was stimulated with the addition of glucose in anoxic soil microcosms (40).

Despite our knowledge of the mechanisms and controls of N cycling and N metabolism, we do not yet fully understand how these genes are regulated within complex soil microbial communities. Metatranscriptomics allows us to capture the transcriptional profile of a microbial community, providing insight into the potential activity of a community at a given moment in time (41–43). Many studies utilizing this technique have focused on the influence of ecosystem

118 level characteristics/properties on transcription, such as land-use, above ground cover,
119 seasonality, and climate (e.g. 38–43). Although these studies contribute greatly to our
120 understanding of community gene transcription, there is additional need to study the dynamic
121 short-term responses of microbial communities to changes in C and N availability (50).

122 In order to fill this knowledge gap, we conducted a soil incubation study where we
123 induced rapid immobilization of inorganic N by adding glucose. We selected glucose as it is a
124 form of labile C commonly found in soils (51), and has been widely used to alleviate C limitation
125 in soil microbial communities as a means to study growth (52, 53) and metabolic activity (50).
126 We analyzed metagenomes and metatranscriptomes of the soil microbial community using high
127 throughput shotgun sequencing to identify the response of N cycling genes over a 48-hour
128 period. We hypothesized that the abundance of N-cycling genes in the metagenomes would not
129 significantly change throughout the course of the 48-hour incubation, but that changes in activity
130 would be immediately detected in the metatranscriptomes. We further hypothesized that there
131 would be an upregulation of genes associated with inorganic N transport, N assimilation into
132 biomass, and N metabolism regulation in response to labile C inputs, and that the abundance of
133 these transcripts would track the concentrations of inorganic N. This work provides an in-depth
134 look at the short-term transcriptional response of soil microbial communities during a central
135 biogeochemical process in soils.
136

METHODS

Soil Sampling and Site Description

Soils were collected in the fall of 2017 from a long-term crop rotation experiment at the West Virginia University Certified Organic Farm near Morgantown, West Virginia, USA (39.647502° N, 79.93691° W; 243.8 – 475.2 m a.s.l.) (54, 55). Samples were taken from plots subject to a four-year conventionally tilled crop cycle consisting of corn, soybean, wheat and a mix of kale and cowpea. Manure was added every two years (during corn and wheat planting), and rye-vetch was added as a winter cover crop before replanting corn in the spring. From each plot, 10 cores 0-10 cm depth were collected and pooled.

Laboratory Incubation

Soil samples were shipped on ice to Northern Arizona University in Flagstaff, Arizona, USA. Soils from 3 plots were pooled, cleaned of roots and large debris, passed through a 2 mm sieve, and distributed between 64 glass Mason jars (500 mL), generating microcosms containing 30 g of soil each. The soil was preincubated at lab temperature (~ 23 °C) for 2 weeks prior to the glucose addition.

The microcosms received 1.6 mL of 0.13 M glucose solution, which added 0.7 mg of glucose C g^{-1} dry soil and raised the moisture content to 60% water holding capacity. Concentrations of glucose in this range have been demonstrated to stimulate soil microbial communities without creating a detrimental increase in osmotic pressure (52). Moreover, a brief trial incubation was conducted to ensure that this concentration of glucose would stimulate CO_2 production. Soils were incubated at lab temperature (~ 23 °C) under ambient lighting, but never direct sunlight. Every 4 hours, over a 48 h period, 5 jars were randomly selected and

destructively sampled. From each jar, we measured headspace CO_2 concentration, concentrations of NO_3^- and NH_4^+, and microbial biomass. A portion of each sample was immediately frozen using liquid N_2 and stored at -80°C for DNA and RNA extraction.

Since the addition of water may stimulate community activity and respiration, especially when starting with very dry soil (56, 57), we measured respiration in a parallel incubation wherein the same volume of water was added without glucose. Headspace CO_2 from these jars was measured and compared against the glucose additions in order to determine the overall effect of glucose and water on microbial respiration.

Biogeochemical Measurements and Analysis

To measure soil NO_3^- and NH_4^+ concentration, 8 g of soil from each destructively sampled jar were added to 40 ml of 1 M KCl solution, shaken for 1 hour, and filtered through Whatman no. 1 filter paper. Extracts were analyzed on a SmartChem 200 Discrete Analyzer (Westco Scientific Instruments, Brookfield, Connecticut, USA). Microbial biomass was measured using an extraction-fumigation-extraction technique (58), consisting of a 0.5 M K_2SO_4 extraction followed by a subsequent K_2SO_4 extraction with the addition of chloroform. The first extraction provided an estimate of the K_2SO_4 extractable organic C and N from each sample, while the second extraction provided an estimate of microbial biomass C (MBC) and N (MBN). Concentrations of extractable organic C and N were measured on a TOC-L (Shimadzu Corp, Kyoto, Japan). The concentration of CO_2 from the headspace of each microcosm was measured using a LI-6262 CO_2/H_2O Analyzer (Licor Industries, Omaha, Nebraska, USA) as described in Dijkstra et al. (2011) (59).

DNA and RNA Extraction and Sequencing

We extracted DNA and RNA just before (t_0) and 8 (t_8), 24 (t_{24}), and 48 (t_{48}) h after glucose addition (n=4). DNA and RNA were extracted using the RNeasy Powersoil Total RNA Kit (Qiagen) according to manufacturer instructions. DNA was separated from RNA using the RNeasy PowerSoil DNA Elution Kit (Qiagen). RNA samples were treated with RNase-Free DNase Set (Qiagen) to remove any DNA. Nucleic acid concentrations were determined with a Qubit fluorometer (Invitrogen, Carlsbad, California, USA), and purity was assessed with a NanoDrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, Delaware, USA). High-quality samples were sent to the Joint Genome Institute (JGI) for sequencing (60). Paired-end, 2 x 151 bp, libraries were prepared using the Illumina NovaSeq platform (Illumina Inc., San Diego, California, USA). Raw sequence reads were uploaded to the JGI genome portal (https://genome.jgi.doe.gov/portal/) under GOLD project ID Gs0135756. A more detailed description of the sequencing can be found in the data release (61).

Metagenome and Metatranscriptomic Analysis

Metatransciptomes were assembled by JGI using MEGAHIT v1.1.2 (62) (parameters "megahit —k–list 23,43,63,83,103,123 —continue –o out.megahit") and metagenomes were assembled using SPAdes version 3.13.0 (63). Assembled metatranscriptomes and metagenomes were uploaded to the Integrated Microbial Genomes and Microbiomes (IMG/M) (64) pipeline for annotation. Full details of the bioinformatics pipeline, as well as SRA reference numbers can be found in the data release (61). From IMG/M we retrieved the number of reads for all genes attributed to functional orthologs in the Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthology database (65), as well as taxonomic annotations against the IMG database. Contigs are

206 available through the JGI genome portal, and taxonomic and functional annotations of these
207 contigs are available on the IMG/M database (http://img.jgi.doe.gov), under GOLD project ID
208 Gs0135756. JGI Genome ID's for each sample, as well as sample metadata, can be found in
209 Chuckran et al (2020; 61).

210 Normalization of KEGG functional annotations was preformed using the Bioconductor
211 (66) program DESeq2 (67) in R. DESeq2 uses a negative binomial distribution to normalize read
212 counts and estimates average $\log_2$ fold change (LFC) between harvests. Significant LFCs for
213 each KEGG functional gene and transcript were determined through both a likelihood ratio test
214 (for overall significance) and Wald test (for specific contrasts between timepoints) provided in
215 DESeq2. Significance for both tests were assumed as a false discover rate (FDR) < 0.01. Prior to
216 analysis, genes with less than 60 reads summed over all samples were discarded in an effort to
217 reduce the FDR correction and improve detection of significant LFCs (68).

218 To assess differences in genes and transcripts composition over time, we performed
219 permutational multivariate analysis of variance (PERMANOVA) on our metagenomes and
220 metatranscriptomes. PERMANOVAs were conducted using Bray-Curtis dissimilarity matrices of
221 the square root transformed normalized read counts with 999 permutations. A SIMPER analysis
222 was used to determine genes which most strongly influenced differences between harvests.
223 PERMANOVAs and SIMPER analyses were conducted using the vegan package (69) for R.

224 To assess the response of N metabolism to the addition of glucose, KEGG Orthology
225 identifiers (K numbers) were grouped according to KEGG pathways and modules associated
226 with N cycling (70), and K numbers representing regulatory genes controlling N metabolism
227 were identified (8, 25) (Table S1). The response of C metabolism was determined by grouping
228 K numbers by KEGG modules associated with glucose uptake, specifically the Entner-Doudoroff

pathway (KEGG module M0008), TCA cycle (M00009), pentose phosphate pathway (M00004), gluconeogenesis (M00003), and Glycolysis (M00001). From the TCA cycle, we also determined the response of isocitrate dehydrogenase (*icd*), which produces oxoglutarate, an important metabolite linking C and N metabolism (32). Counts and LFCs for K numbers were then averaged for each module to assess the overall response for each process. Results were visualized using the ggplot2 package (71) in R v 3.6.1 (72).

RESULTS

Biogeochemical Measurements

The concentration of NO_3^- decreased in the 24 hours after glucose addition and remained low for the remainder of the incubation (Fig. 1A). The concentration of NH_4^+ also decreased during the first 24 hours of the incubation and increased thereafter (Fig. 1B). Rates of CO_2 production increased from 4-16 hours and then decreased from 28-48 hours in response to glucose (Fig. 1C). We found that the addition of water only slightly influenced CO_2 production (Fig. S1), indicating that the majority of the stimulation was due to the addition of labile C. K_2SO_4 extractable organic carbon decreased for the first 20 hours and plateaued thereafter (Fig. 1D). Based on these biogeochemical measurements, we selected 4 timepoints (t_0, t_8, t_{24}, and t_{48}) from which we extracted DNA and RNA. These timepoints captured distinct phases of C and N availability that enabled us to test our hypotheses.

Microbial biomass C (MBC) moderately decreased throughout the incubation (Fig. S2A) and microbial biomass N (MBN) remained constant (Fig. S2B). Bacteria may exhibit some stoichiometric plasticity in response to nutrient inputs (73), however a decrease in biomass C:N in response to C inputs is counter-intuitive. Since the method of microbial biomass extraction used involves two extractions on the same sample (one before and after fumigation), incomplete extraction of the added glucose in the first extraction could result in an artificially high estimate of biomass C. We believe that it is far more likely that microbial biomass and stoichiometry did not change, and that changes in estimated MBC are likely the result of unextracted glucose remaining from the initial K_2SO_4 extraction.

Metagenomic and Metatranscriptomic Assembly and Annotation

Out of 16 soil samples from which DNA and RNA were extracted, 12 were successfully sequenced and assembled for metagenomic analysis and all 16 for metatranscriptomic analysis. For the metagenomes, the proportion of genes successfully annotated against the KEGG database varied from 23.4% to 25.6% of all genes per sample. Of the 6,876 functional KEGG orthologs identified in the metagenome analysis, 671 genes were in higher abundance while 332 were present in lower abundance (FDR < 0.01) after the addition of glucose. Glucose caused a shift in the relative abundance of functional genes (PERMANOVA, $F_{3.11} = 3.24$, $P < 0.01$; Fig. 2A). The genes that were most different in gene abundance relative to t_0 varied for each timepoint (SIMPER analysis; Table S3A), and not one of these genes was directly related to N uptake. Among these were the subunits of RNA polymerase *rpoB* and *rpoC,* which were in slightly lower abundance at t_8 (LFC -0.1, FDR > 0.1), and the regulatory gene for the lac operon, *lacI*, which was in a greater abundance at t_{24} and t_{48} (LFC 0.7, FDR < 0.01). The largest changes were found at t_{24} for low-abundant spore gemination proteins (Table S3B), specifically *gerKC* (KO6297) and *yfkQ* (K06307) which were 8.8 and 7.4 LFC more abundant than at t_0.

The proportion of transcripts successfully annotated against the KEGG database varied between 12.6% and 32% of all transcripts in a metatranscriptome. Transcripts for 5,448 functional genes were identified, of which 1,141 increased and 855 decreased in response to glucose. A PERMANOVA indicated significant shifts in the abundance of transcripts between timepoints ($F_{3, 15} = 8.07$, $P < 0.01$; Fig. 2B). Transcripts encoding for *amt* and *glnA* contributed the most to dissimilarity with t_0 (SIMPER analysis), combined they explained 1% of the differences at t_8, 1% of differences at t_{24}, and 0.9% of differences at t_{48}.

Gene and Transcript Abundance of Nitrogen Cycling Processes

The abundance of N cycling genes was generally stable over time (Fig. 3A), with changes in gene abundance often being several orders of magnitude smaller than changes in transcript abundances. For metatranscriptomes, many genes associated with N uptake were highly upregulated in response to glucose (Fig. 3). Expression of genes encoding the GS-GOGAT pathway (GS - *glnA;* GOGAT - *gltS, gltD, gltB*) was consistently upregulated after glucose addition (FDR < 0.01), peaking at 8 h (Fig. 3B, Table S2). We did not find a similar trend for transcripts associated with glutamate dehydrogenase (GDH: *gudB, gdhA*). Instead we found variable increases and decreases in the expression for these genes which corresponded with different classes of GDH enzymes (Fig. 3B Table S2). In prokaryotes, GDH often uses NADH (EC 1.4.1.2), NADPH (EC 1.4.1.4) as cofactors, while GDH in eukaryotes can use both (NAD(P)H; EC 1.4.1.3) (74). Transcription of genes for EC 1.4.1.4 significantly increased early ($t_{8,}$ LFC 1.542 ± 0.312; FDR < 0.01), and transcription for EC 1.4.1.2 trended higher later (t_{48}, LFC 2.229 ± 0.884; FDR < 0.1). The eukaryotic EC 1.4.1.2 gene GDH2 (K15371) was upregulated at t_{24} (LFC 1.350 ± 0.434; Table S2; FDR < 0.01) and EC 1.4.1.3 was slightly downregulated throughout (significantly at t_8, FDR < 0.01).

The abundance of transcripts encoding the ammonium transporter AmtB (*amt*) was significantly (FDR < 0.01) higher after glucose addition throughout the 48-h incubation (Fig. 3B, Table S2), peaking at t_8, where it was 16-fold higher than at t_0 (41,366 transcripts at t_8 vs 2,539 at t_0). A similar upregulation was found for genes associated with nitrate and nitrite transport across the membrane – 1500-fold increases compared to t_0 (from 2.6 to almost 2800 transcripts per sample at t_{24}; Fig. 3B).

Genes associated with assimilatory nitrate reduction (Fig. 3; Table S2) were strongly upregulated at t_8 and remained upregulated over the 48 h incubation period. In contrast, we found

variable responses of genes associated with DNRA. Most genes associated with the dissimilatory reduction of nitrate to nitrite were downregulated or not significantly affected, with a few exceptions. Nitrate reductase gamma subunits (*narI/narV*) were upregulated at t_{24} and t_{48}, and the genes *nirB* and *nirD*, which encode the small and large subunit of the cytosolic enzyme nitrite reductase, were significantly (FDR < 0.01) upregulated throughout the incubation (LFC 6.18 to 7.70; Fig. 3B). In contrast to these enzymes, abundance of transcripts that encode a periplasmic cytochrome c nitrite reductase (*nrfA* and *nrfH*) did not significantly change in response to C amendment.

Expression of all genes involved with nitrification were downregulated in response to glucose, and a majority of those genes (5 of 6) were significantly (FDR < 0.01) downregulated at some point during the incubation (Fig. 3B). Similarly, expression for most denitrification genes were downregulated throughout the incubation, with the exception of *narI* and *narV*, which encode for gamma subunits of nitrate reductase.

Transcripts for three genes that encode subunits of nitrogenase (*nifK*, *nifD*, and *nifH*) were detected, all of which were at very low abundance at t_0, t_8, and t_{48}. Only at t_{24} did we observe a strong significant (FDR < 0.01) upregulation for all 3 genes, up to 410-fold higher than t_0 for *nifH* (798 transcripts at t_{24} vs 1 at t_0; Fig. 3B).

We found that the vast majority of N cycling gene transcription could be attributed to bacteria and archaea (Fig. 4). Dissimilatory processes were largely from *Thaumarchaeota* and *Nitrospirae*, while assimilatory processes tended to be represented by *Proteobacteria*, *Actinobateria, and Acidobacteria*. Nitrogen fixation was heavily dominated by *Proteobacteria* (Fig. 4).

328 *Regulation of N Cycling Genes*

329 Generally, transcripts of genes associated with regulation of N metabolism increased after
330 glucose addition (Fig. S3; Fig. 5). The abundance of ATase and UTase (*glnD* and *glnE),* used for
331 post-modification of glutamine synthetase (GS) and regulatory PII proteins respectively, initially
332 increased at t_8 (2.18 ± 0.41 LFC and 4.31 ± 0.36 LFC; FDR < 0.01; Fig. S3; Fig. 5). UTase
333 (*glnD*) but not ATase (glnE), continued to be significantly upregulated at t_{24} (3.79 ± 0.36 LFC)
334 and t_{48} (2.75 ± 0.36 LFC; Fig. S3). Similar upregulation was noted for PII proteins GlnB (*glnB*;
335 LFC > 2.9; FDR < 0.01; Fig. S3) and GlnK (*glnK*; LFC > 3.9; FDR < 0.01; Fig. S3), and the
336 NtrC family genes *glnL* (FDR < 0.01) and *glnG* (FDR < 0.01 at t_8 and t_{24}; Fig. S3). No
337 significant changes in transcript abundances were found for the transcriptional regulators *nac*
338 *and lrp,* while *crp* and *rpoN* were slightly downregulated (LFC < -1) at t_8 and t_{24} (FDR < 0.01;
339 Fig. S3; Fig. 5).
340
341 *C Metabolism*
342 The LFC and total number of normalized transcripts for processes involved with glucose
343 breakdown (KEGG modules M00001, M00003, M00004, M0008, and M00009). increased from
344 t_0 to t_8 and t_{24} (Fig. S4; Table S4; Tukey's HSD $p < 0.05$). Significant changes in transcript
345 abundance after glucose amendment were found for the Entner-Doudoroff pathway and TCA
346 cycle, including the enzyme isocitrate dehydrogenase (*icd*) which produces oxoglutarate, a
347 metabolite which directly connects C and N metabolism (Fig. 5; Fig. S4B, Table S4).
348

DISCUSSION

Over a period of 48-hours after glucose addition we observed a substantial decrease in K_2SO_4 extractable organic C, an increase in CO_2 production rate, and an increase in the abundance of transcripts for genes associated with glucose breakdown. These changes coincided with a decrease in inorganic N and an increase in the transcript abundance of genes involved with inorganic N uptake, assimilation, and N metabolism regulation. These results demonstrate that soil microbial communities respond to labile C not only by upregulating genes associated with C metabolism, but also by rapidly increasing the transcription of genes responsible for N acquisition. Further, we found that genes for several forms of N acquisition (e.g., N fixation, assimilatory nitrate reduction, ammonium transport) were differentially transcribed over the 48 h incubation, indicating that changes in multiple microbially mediated N transformations occur within this small temporal window.

Inorganic N Uptake and Assimilation

The GS-GOGAT pathway appeared to be the predominant pathway through which ammonium was assimilated into biomass. The other main avenue of ammonium assimilation into biomass, the enzyme GDH, did not show a similar increase in transcript abundance and the abundance of GDH transcripts was substantially smaller than that of GS-GOGAT. This suggests that GS-GOGAT may be the dominant pathway for assimilation of inorganic N in soil microbial communities responding to labile C inputs. This finding is consistent with the notion that GDH is most active when NH_4^+ concentration is high and availability of C is low (27). Assays from soil microbial communities have also shown that GS activity increases in response to higher C to N ratios whereas GDH activity decreases (75). Further, we found that regulation of GDH

372 transcription appeared to be gene specific, with transcription for EC 1.4.1.4 increasing early and
373 EC 1.4.1.2 increasing late. These results nicely follow concentrations of NH_4^+, as NADPH
374 specific enzymes (EC 1.4.1.4) are generally used for ammonium assimilation (76) whereas
375 NADH specific enzymes (EC 1.4.1.2) are commonly used for breakdown of glutamate to
376 ammonium (77). These findings highlight the potential utility of measuring GDH and GS-
377 GOGAT gene transcription for tracking the C and N balance within microbial communities at a
378 given moment in time, which could be a useful approach when, for example, assessing how
379 specific land use practices influence microbial metabolism and N cycling.

380 Various mechanisms for transporting inorganic N across the cell membrane were
381 upregulated in response to glucose inputs. Notably, the gene *amtB*, which encodes for the
382 ammonium transporter AmtB, was the second most abundant upregulated gene during the
383 incubation (behind *glnA*). Similarly, we observed an upregulation of genes associated with
384 nitrate and nitrite transport (KEGG module M00615) and assimilatory nitrate reduction, which
385 coincided with a precipitous drop in the concentration of NO_3^-. Most genes involved with
386 DNRA were not differentially expressed, indicating that nitrate reduction was primarily
387 occurring under aerobic conditions. A notable exception were the genes *nirB* and *nirD*, which
388 encode for the cytosolic enzyme nitrite reductase NirBD (78), which has been shown to be active
389 in aerobic soils (79, 80) and may function as the nitrite reductase in assimilatory nitrate reduction
390 (81). Although the upregulation of N transport genes in response to glucose is certainly not
391 novel (30), these results are the first demonstration of this response in a soil microbial
392 community metatranscriptome. Further, these responses show the short timeframes (within 8 h)
393 in which soil microbial communities can respond to changes in C and N availability.

394 The finding that glucose addition strongly upregulated genes encoding for nitrogenase,
395 especially when NH_4^+ concentrations were low, is consistent with the idea that nitrogen fixation
396 increases when N concentrations are low (82). N fixation has been shown to be activated by the
397 addition of other limiting nutrients such as carbon or phosphorous (83, 84). We therefore believe
398 that the upregulation of nitrogenase genes is a response to low concentrations of NH_4^+ and
399 availability of labile C. The prompt upregulation, and subsequent downregulation, of nitrogenase
400 genes also suggests that some portion of biological nitrogen fixation occurs rapidly in soils, or at
401 the very least that the process is highly sensitive to concentrations of NH_4^+.
402
403 *Connections Between C and N Metabolism*
404 Interestingly, transcripts associated with NH_4^+ and NO_3^- transport maintained their high
405 abundances despite concentrations of NO_3^- stabilizing and concentrations of NH_4 increasing (24-
406 48 h into the incubation). One possible explanation is that the activity of these proteins is
407 dictated through allosteric regulation which is tightly connected to the activity of both C and N
408 metabolism (Fig. 5). For example, the ammonium transporter AmtB is allosterically inhibited by
409 the PII protein GlnK which is indirectly controlled by internal concentrations of glutamine, an
410 intermediate of N uptake through GS-GOGAT (Fig. 5), and oxoglutarate, an intermediate of the
411 TCA cycle (Fig. 5; 29, 66). In this way, internal concentrations of metabolites from both C and N
412 metabolism may dictate N uptake.
413 The transcription of N regulatory genes reflects the importance of intermediate
414 metabolites in regulation. We found that abundance of transcripts for transcriptional regulators
415 (such as *nac*, *lrp*, and *crp*) and σ^{54} were either not affected or slightly reduced (Fig. 5). In
416 contrast, transcripts for genes in the phosphorylation cascade, which links C and N metabolism

417 through intermediate metabolites, were more abundant after the addition of glucose (Fig. 5). The
418 upregulation of the two component regulatory NtrB (*glnL*, *ntrB*) and NtrC (*glnG*, *ntrC*) within
419 this cascade is especially noteworthy, as this system regulates ~75 genes associated with N
420 acquisition, including glutamine synthetase (Fig. 5) (86).

421 Since the activity of this regulatory network is tightly controlled by internal
422 concentrations of metabolites (30), it is not possible to determine the activity of many of these
423 proteins through the metatranscriptome alone. However, it is noteworthy that almost all of the
424 genes within this regulatory network were upregulated, even if the encoded protein potentially
425 inhibited N transport or assimilation (e.g. GlnK; Fig. 5). This broad upregulation of genes in the
426 phosphorylation cascade may be beneficial during C uptake, as it allows the concentration of
427 nutrients and metabolites to control N uptake, thereby ensuring N uptake matches the supply of
428 C (25, 32).

429

430 *Nitrification and Denitrification*

431 Most genes associated with nitrification and denitrification were significantly
432 downregulated. Since nearly all nitrifiers in this soil were autotrophic archaea (55), this finding is
433 consistent with the premise that addition of glucose reduces rates of autotrophic nitrification by
434 reducing the amount of available ammonium (37). It is not especially surprising that we did not
435 find an upregulation of denitrification genes, as denitrification is most prevalent in anoxic
436 systems with high availabilities of nitrate.

437

438 *Genetic Potential Versus Transcription*

Notably, although we did observe a slight shift in the functional composition of our metagenomes, these changes did not track those found in the metatranscriptomes in either magnitude or direction. Changes contributing the most to dissimilarity tended to be slight shifts in highly abundant genes, such as *rpoB*, *rpoC*, and *lacI*. We found interesting differences in the abundance of spore forming proteins as nutrient availability declined, however since many of these proteins were uncommon and in low abundance, the chance of obtaining a false positive is much greater and we are therefore cautious to draw any conclusions based on these data alone. Changes in gene abundance for most N cycling genes were absent. These results suggest that understanding the response of soil microbial communities to short-term changes in the environment necessitates looking beyond the metagenome, as consequential microbial responses occur through changes in gene-expression. This is in line with other studies where the composition of transcripts shifts over hours or days (12, 87), whereas shifts in metagenomic community composition have been shown to occur after weeks or months (72) .

Our work represents a preliminary look into the short-term transcriptional response of microbial communities in response to a change in C availability, however there are a number of considerations moving forward. More work needs to be done focusing on this response in a variety soils, as nutrient availability and other soil properties will undoubtably influence this process. For example, soils high in C and low in N would likely not demonstrate a similar response as observed for this agricultural soil. Understanding how ecosystem properties influence the dynamics of transcriptional profiles is therefore necessary in determining short-term microbial contributions to biogeochemical cycling. Further, this work focused on a relatively short timeframe, however whether this increase in transcription persists or influences nutrient cycling on the scale of weeks to months remains to be seen. Finally, future efforts should

462 be made to observe these short-term effects *in situ*. Laboratory incubations are extremely useful
463 for controlling environmental variables and isolating a particular response. However, it is likely
464 that under field conditions, and in the presence of plant roots, factors other than C availability
465 will affect the gene-expression at the same time and to different degrees, potential masking the
466 response observed in this short-term laboratory experiment.
467
468

CONCLUSIONS

Our results indicate strong and rapid upregulation of genes associated with uptake of inorganic N, assimilatory nitrate and nitrite reduction, GS-GOGAT pathway, and the regulatory network underlying N cycling. Further, the majority of upregulation occurred in pathways which are largely aerobic and heterotrophic, suggesting that these processes dominate the short-term response to labile C in these soils. Perhaps most importantly, this work highlights the importance of microbial gene transcription in controlling short-term biogeochemical cycling in soils. Within the 48 h incubation we found that microbially mediated transformations of N were well reflected in the metatranscriptome but not in the metagenome or in microbial biomass. The short-term transcriptional responses of soil microbes may therefore serve an important role in determining how biogeochemical fluxes respond to immediate changes in the environment.

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496

497 Competing interests:

498 The authors have no competing interests to disclose

499

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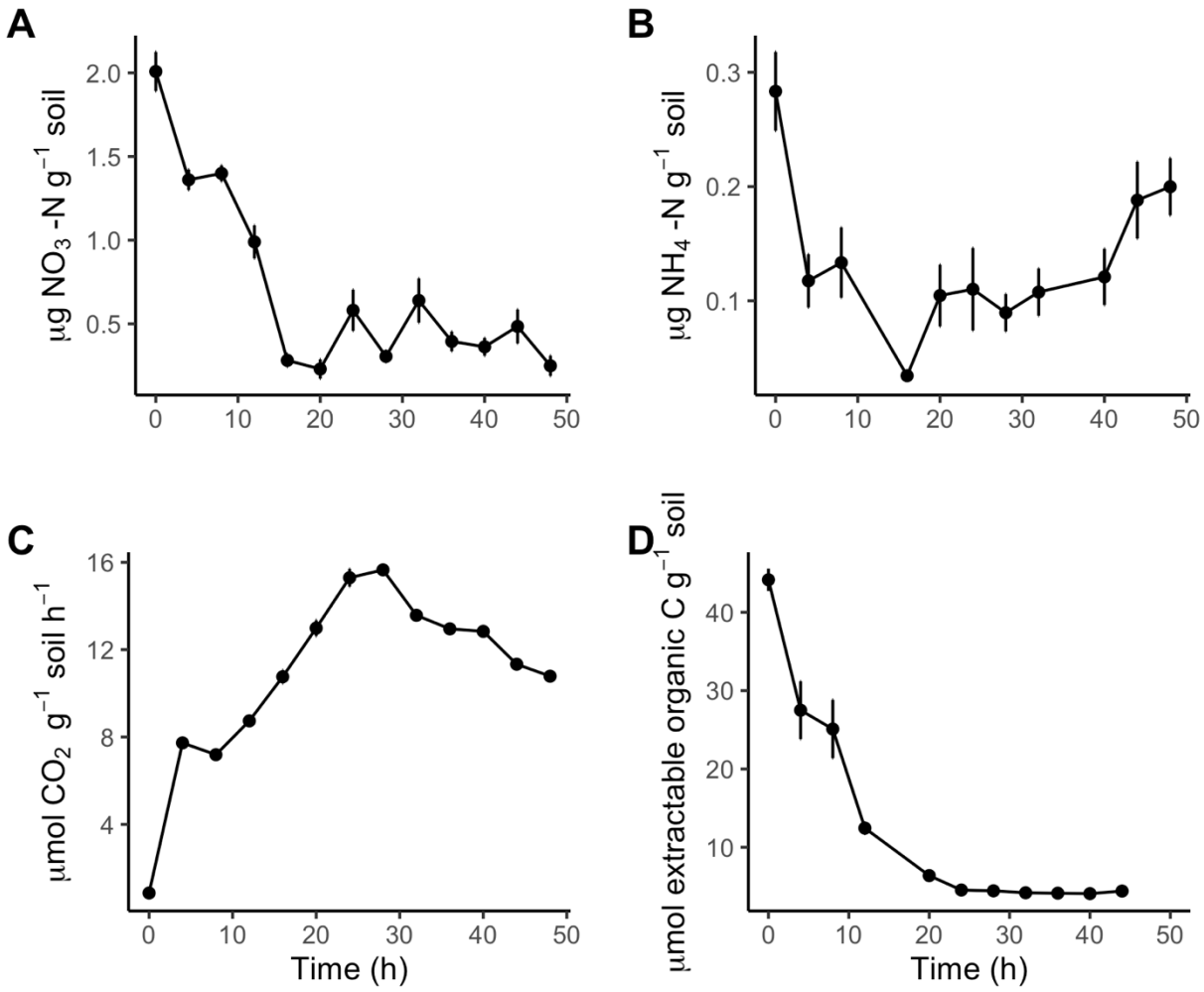
730 1480.

731

732 FIGURES

733 **Figure 1.** Mean concentration (± SE) of nitrate (**A),.** ammonium **(B)**, rate of carbon dioxide

734 production **(C)**, and K_2SO_4-extractable C **(D)** as a function of time after glucose amendments.



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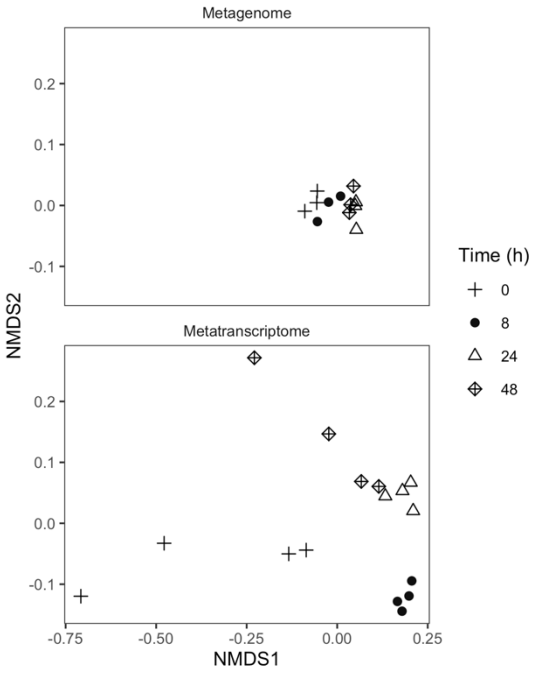
737 **Figure 2.** NMDS using Bray-Curtis distance of normalized KEGG annotation abundance for
738 metagenomes (**A**) and metatranscriptomes (**B**) at 0, 8, 24, and 48 hours after the addition of
739 glucose.



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743 **Figure 3. (A)** Log_2-fold changes (mean LFC ± SE) relative to t_0 of normalized gene (left) and
744 transcript (right) abundances versus normalized counts for N cycling genes from glucose-
745 amended soils. LFC and normalized counts represent the average between $t_{8,}$ t_{24}, and t_{48} for each
746 gene. **(B)** Log_2-fold changes in transcript abundances for genes grouped by biologically relevant
747 reactions and pathways. A black asterisk indicates a significant change relative to t_0.
748

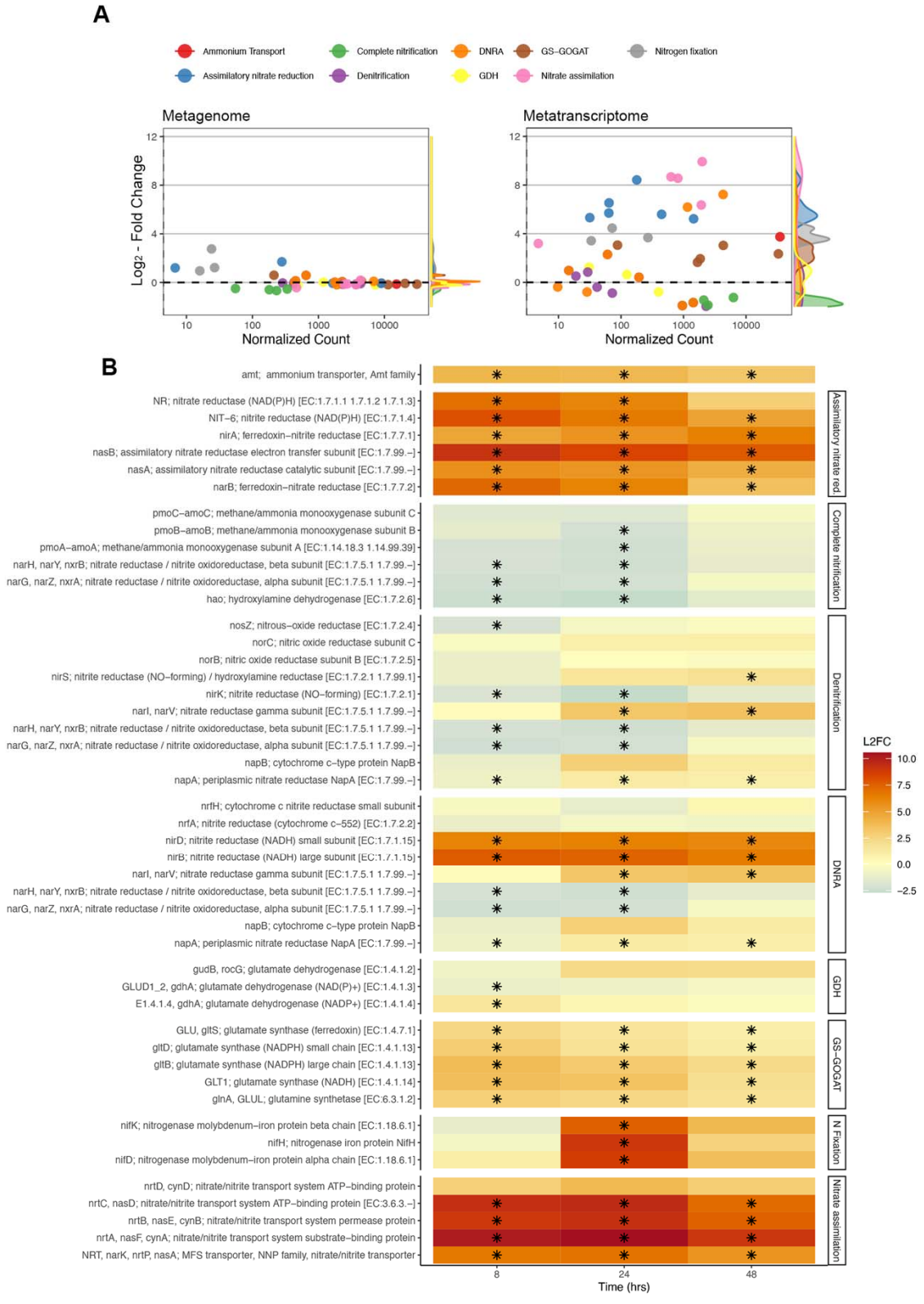
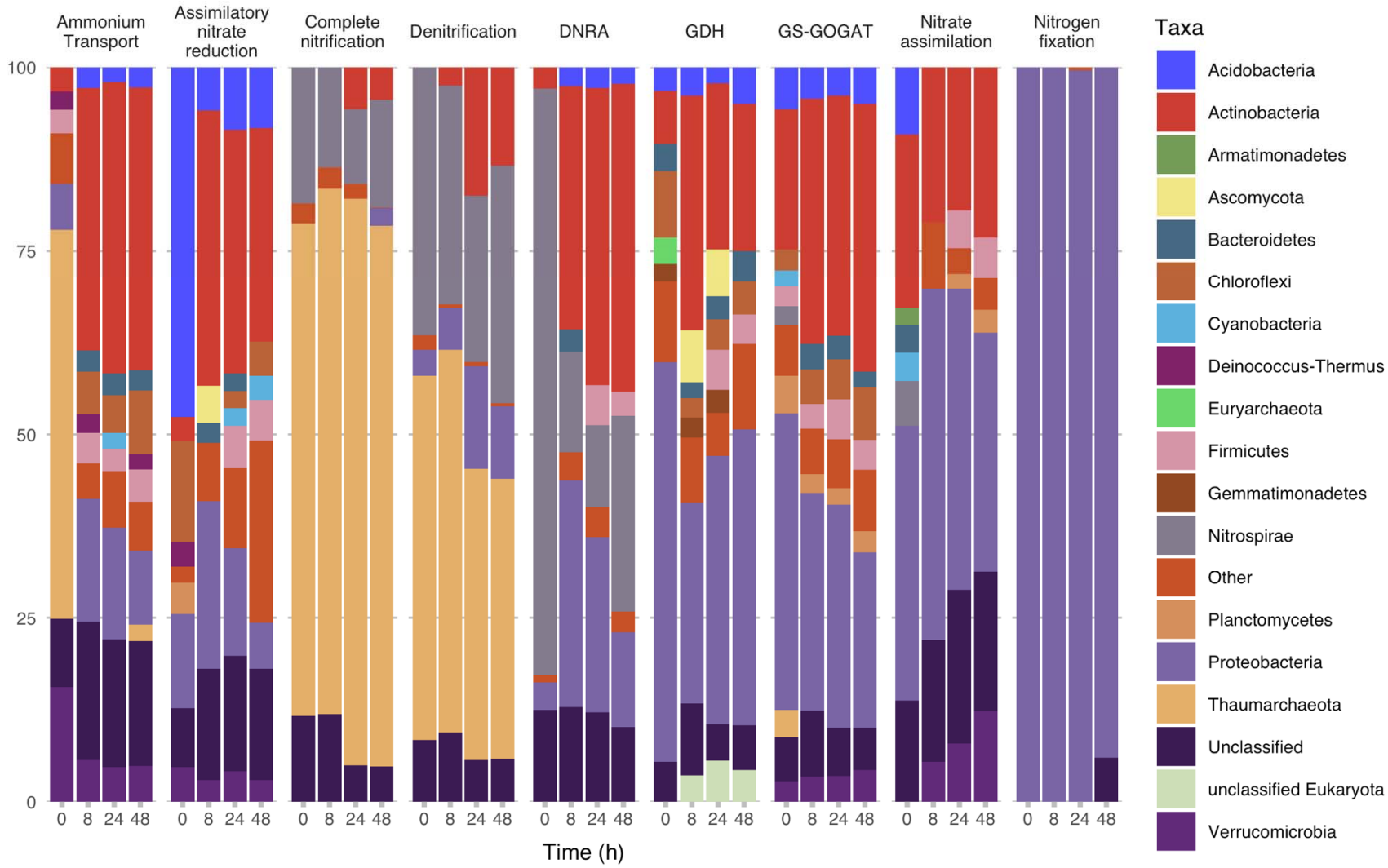
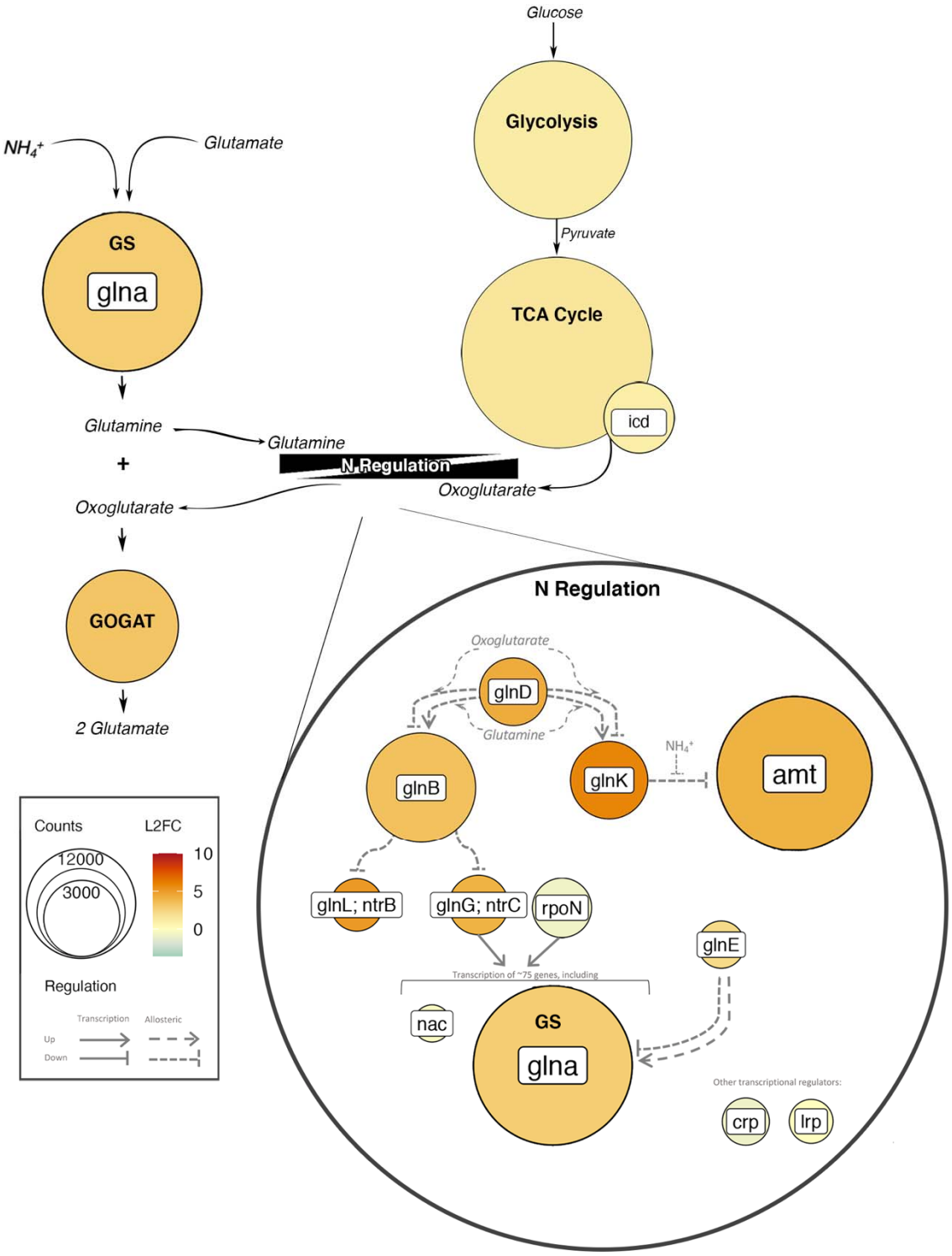


Figure 4. Relative transcript abundance of major taxa for reactions and pathways of N-cycling at 0, 8, 24, and 48 hours after glucose amendments.



754 **Figure 5.** Abundance and log_2-fold change of transcripts 8 h after glucose addition of C and N
755 metabolism including glycolysis, the TCA cycle, N regulatory network, and GS-GOGAT. Color
756 represents log_2-fold change of transcript abundances relative to t_0, and size indicates number of
757 transcripts. Thin black arrows indicate reactants or products of pathways and grey arrows
758 represent regulatory controls. Gene names are presented in white boxes (ex. *glnA*), whereas
759 pathway or enzyme names are presented in bold (ex. GS or Glycolysis).



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