AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2019 – 2021

TITLE: Response of soil N mineralization to temperature

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EXECUTIVE SUMMARY: Information about the response of soil respiration, N mineralization, and nitrification across temperatures is relevant to Oregon agriculture, and will allow the development of chemical equilibrium microbial kinetic (CEMK) models to predict the response of C and N processes and rates of NH_4^+ mineralization to changes in temperature regimes in other soils across the region. The data collected, and the model developed during this study will be used to justify funding from USDA/NIFA or NSF to evaluate the effects of climate uncertainty on crop available N.

OBJECTIVES: We will evaluate soil C and N cycle coupling during constant temperature incubations at seven temperatures (4 – 42°C). We will utilize soils from major cropping areas in western and eastern Oregon. Within each sampling area we will identify soils of the same soil type under conventional management (low soil C), and soils managed to increase soil C content (high soil C). The temperature response of net N mineralization, CO_2 respiration, and nitrification in each soil will be modeled to obtain thermodynamic parameters to compare the different processes. The obtained thermodynamic parameters will be used to inform chemical equilibrium microbial kinetic (CEMK) model to predict the response of the different processes to changes in temperature regimes. We predict distinct differences in rates of respiration and inorganic N accumulation across the incubation temperature range.

PROCEDURES: Soils from two major Oregon agroecological zones were collected in June and July of 2019. Soils from OSU's Columbia Basin Agricultural Research Center (CBARC) in Pendleton Oregon are representative of the intermountain cereal cropping region of the Pacific Northwest that has a mean annual temperature (MAT) of 11.2°C (temperatures range -7.2 – 38.3°C), and receives ≤ 18 inches of precipitation annually. Pendleton soils are Typic Haploxerolls (Walla Walla silt loams) in a winter wheat/fallow rotation in a long-term tillage fertility study since 1940. Three replicates of tilled (annual tillage, pH 5.5 – 6.6) and No-till (pH 4.9 – 5.1) soils were sampled from the randomized block, split plot treatments receiving 80 lbs N per year. Four to five soil samples were taken to a depth of 10 cm from each replicate plot via a random walk process. A composited sample was prepared for each replicate plot and brought to the laboratory.

We collaborated with Kristin Trippe, a USDA Research Microbiologist at the Forage Seed and Cereal Research Lab in Corvallis, to find a pair of soils from the Willamette Valley under conventional tillage (low soil C) and more conservative tillage management (higher soil C). The Willamette Valley sites have a MAT that is not significantly different from the Pendleton site (11.5 ^{o}C); however, Willamette Valley soils rarely freeze and experience a lower maximum temperature (temperature range 3.3 – 33.9^{o}C). The Willamette Valley receives an average of 42 inches precipitation yearly, and soils on the valley floor are often water saturated during winter and spring, and commonly used for crops tolerant to wet soil conditions such as spring grains, grass seed and pasture. Soils for this study were sampled from annual rye grass fields belonging to a private grower under Till (annual tillage and residue removal, pH 4.7 – 5.8) and No-till (infrequent tillage and receiving full residue load, pH 6.5 – 7.3) management, and receive 140 lbs. N acre^{-1} year^{-1}. Both fields were on Dayton series soils (Vertic Albaqualfs) which are widely distributed in the Willamette Valley. The fields were large (70 – 80 acres each) and were sampled to yield three distinct field replicates. Four to five soil samples were taken to a depth of 10 cm from each replicate site via a random walk process, and a composited sample was prepared for each replicate site and brought to the laboratory. In the laboratory all soils were sieved to <4.75 mm and stored at 4^{o}C prior to experimentation.

Field replicates of high and low C soils from each sampling area were incubated moist (field capacity) at seven temperatures (4, 10, 16, 23,30, 37, 42^{o}C) under three treatments 1) plus acetylene (10 µM concentration in soil water, C_{aq}) as a negative control for nitrification activity allowing the determination of net NH_4^+-N mineralization, 2) plus octyne (4 µM C_{aq}) to discriminate between nitrification by AOA and AOB as described by Taylor, Vajrala et al. (2013), and 3) no alkyne treatment as a positive control for nitrification and respiration. Incubations were sampled weekly over the course of one month. At the beginning and end of each incubation total N and C were determined to evaluate the soil C and N lost from the soil. At each sampling time headspace gas was analyzed for carbon dioxide (CO_2) to determine rates of respiration. Soil samples were extracted for NH_4^+, NO_2^- and NO_3^- and rates of NH_4^+ mineralization and nitrification by AOA and AOB determined.

The temperature response rate data has been modeled using the macromolecular rate theory (MMRT) to obtain thermodynamic parameters for the CEMK predictive model. We intend to extend our previous model of the interactions of NH_3-oxidizing and NO_2^- oxidizing bacteria to soil N-cycling processes (Mellbye, Giguere et al. 2018).

SIGNIFICANT ACCOMPLISHMENTS TO DATE: The incubations and much of the analysis has been completed. One manuscript is in review at Frontiers of Microbiology. A second manuscript is being finalized and should be submitted by Summer of 2021. Highlights from the two manuscripts are included here.

Manuscript 1: Implications of the thermodynamic response of soil mineralization, respiration and nitrification on soil organic matter retention

Considerable research has shown that modifications in global temperature regimes can lead to changes in the interactions between soil respiration and the sequestration of C and N into soil organic matter (SOM). We hypothesized that despite the interconnected nature of respiration, net N mineralization, and nitrification processes, that there would be differences in their thermodynamic responses that would affect the composition of inorganic soil N and the potential for retention of N in SOM. To test this hypothesis, soil respiration, N mineralization and nitrification responses were evaluated during constant temperature incubations at seven temperatures (4 – 42^{o}C) in tilled and no-till soils from two major agroecological zones in Oregon; Willamette Valley, and Pendleton located in the Columbia River Basin. We observed (1) significant thermodynamic differences between the three processes in all soils, (2) a distinctly different thermodynamic profile in Willamette vs. Pendleton, and (3) a dynamic response of T_{opt} (optimal temperature for activity), and T_{smax} (temperature of greatest rate response to temperature), and temperature sensitivity ($\Delta C_p^{\ddagger}$) over the incubation time course, resulting in shifts in the thermodynamic profiles that could not be adequately explained by changes in process rates. We found that differences in contributions of ammonia oxidizing archaea and bacteria to nitrification activity across temperature helped to explain the thermodynamic differences of this process between Willamette and Pendleton soils. A two-pool model of SOM utilization demonstrated that the dynamic thermodynamic response of respiration in the soils was due to shifts in utilization of labile and recalcitrant pools of C; and that the respiration response by Pendleton soils was more dependent upon contributions from the recalcitrant C pool resulting in higher T_{opt} and T_{smax} than Willamette soils. Interestingly, modeling of N mineralization using the two-pool model suggested that only the recalcitrant pool of SOM was contributing to N mineralization at most temperatures in all soils. The difference in labile and recalcitrant SOM pool utilization between

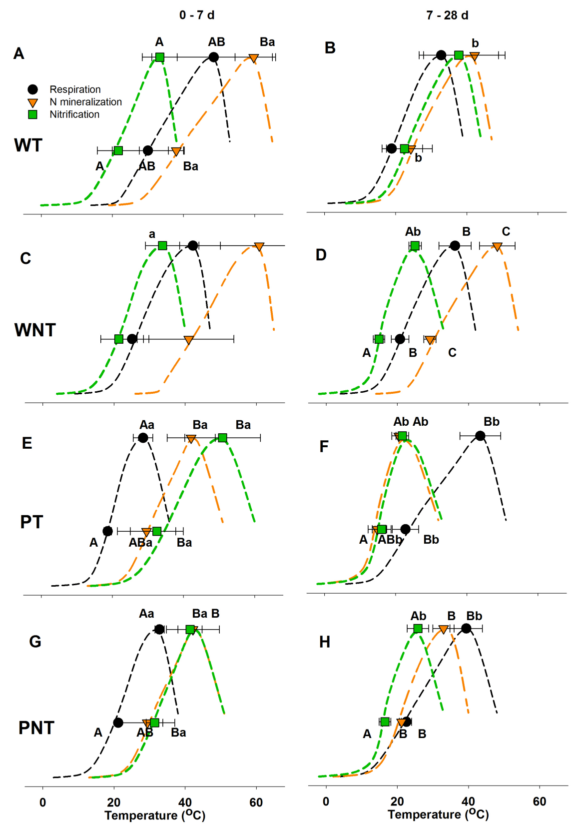

Figure 1. Comparison of thermodynamic characteristics of respiration, net N mineralization and net nitrification over 0 – 7 d (A, C, E & G) and 7 – 28 d (B, D, F & H). The symbols represent the average value of the thermodynamic parameters determined for three field replicates of each of the four sampled soils, and the error bars represent the standard deviation of the average. The dashed lines are presented to aid the visualization of the temperature response with an inflection point showing maximum sensitivity to temperature change (T_{smax}) and the optimal temperature (T_{opt}). Upper case letters indicate significant differences in T_{smax} or T_{opt} between the three processes within a time interval; and lower case letters indicate significant difference in T_{smax} or T_{opt} of an individual processes between the two time periods ($p \leq 0.05$).

respiration and N mineralization may suggest that these processes may not be as interconnected as previously thought.

Manuscript 2: The effect of temperature on availability of mineralized inorganic N

There were significant correlations between C and N mineralization in all four soils ($r \geq 0.607$, $p \leq 0.01$). In Willamette soils, the accumulation of net NO_3^- + NH_4^+ over 28d responded significantly to temperature increase (Figure 2C). Concentrations of net NO_3^- + NH_4^+ increased significantly above 4°C until maximum N mineralization was achieved at 37°C in No-till soil and 37 and 42°C in Tilled soil. In Willamette No-till soil the accumulation of NO_3^- + NH_4^+ at 42°C was significantly less than at 37°C ($p \leq 0.05$). The rate of net N mineralization over 4 – 37°C in Willamette soils could be adequately described by an exponential function (Figure 2C and 2D). Despite there being ~2-fold higher rates of respiration at all temperatures in Willamette No-till soil than in Tilled soil (Figure 1A), as well as having significantly more Total N, the only significant difference in net NO_3^- + NH_4^+ accumulation between Willamette soils was at 37°C where No-till soil had ~2-fold higher concentration of NO_3^- + NH_4^+. This may suggest that in the Willamette No-till soil at temperatures ≤ 30°C that a significant amount of NH_4^+ was immobilized into biomass. This possibility is supported by an evaluation of the C:N ratio of accumulated CO_2-C and total mineralized N (NO_3^- + NH_4^+). We reasoned that in the absence of immobilization of N by soil microbes, that the accumulated CO_2-C:inorganic N should be similar to the soil C:N. However, we saw that in Willamette No-till soil an inflated C:N of accumulated CO_2-C:inorganic N (17±4 to 30±8) at 10, 17, 23 and 30°C compared to soil C:N (11.9±0.5) which could indicate that NH_4^+ was immobilized into microbial biomass. In Willamette Tilled soil the

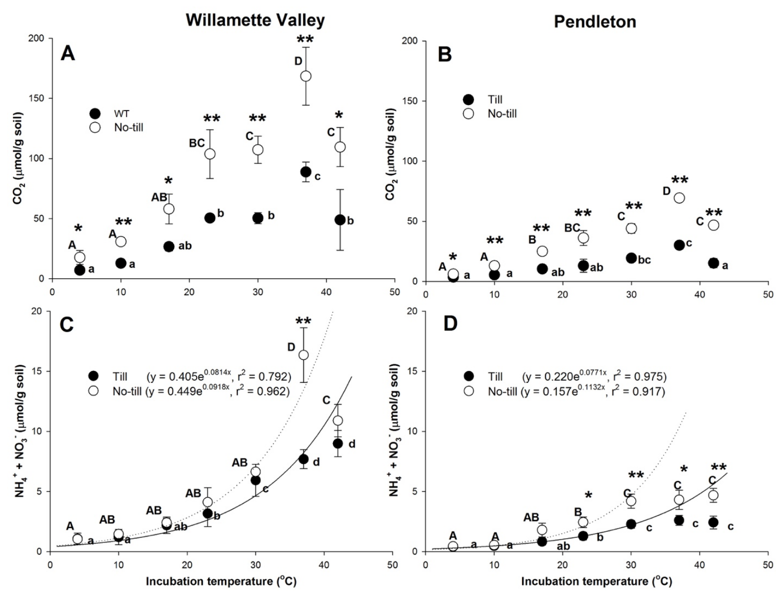

Figure 2. Respiration and N mineralization response across temperatures. The accumulation of CO_2 and net N mineralization (NO_3^- + NH_4^+) in Willamette (A and C) and Pendleton (B and D) soils over 28d under Till and No-till management. Lower case and upper case letters indicate a significant difference (ANOVA $p \leq 0.05$) in CO_2 or net N mineralization accumulated at each temperature in Tilled or No-till soils, respectively. Asterisks indicate a significant difference between Till and No-till treatments as determined by a two-tailed ttest; *$p \leq 0.05$, **$p \leq 0.01$. CO_2 accumulation was always significantly greater in Willamette soil than in Pendleton soil of the same management type ($p \leq 0.05$). Equations and lines describe the exponential accumulation of net NO_3^- + NH_4^+ in response to temperature.

C:N of accumulated CO_2-C:inorganic N did not differ significantly from the soil C:N at any temperature.

In both Pendleton soils, the accumulation of net NO_3^- + NH_4^+ over 28d responded significantly to temperature ($p \leq 0.05$, Figure 2D), with increases in NO_3^- + NH_4^+ accumulation up to 30°C, after which levels of mineralized NO_3^- + NH_4^+ did not change. Maximum NO_3^- + NH_4^+ accumulated in both Till and No-till Pendleton soils at 30, 37 and 42°C. An exponential function could describe the rate response of net N mineralization over 4 – 30°C in Pendleton soils (Figure 2C and 2D). Pendleton No-till soils had significantly higher CO_2 accumulation over 28 d, and greater total soil N than Tilled soils, and significantly more NO_3^- + NH_4^+ accumulated in Pendleton No-till soil than Pendleton Tilled soil at temperatures ≥ 23°C. In Pendleton No-till soil the C:N of accumulated CO_2-C:inorganic N at 10 and 23°C (36±4 and 16±0.2, respectively) were significantly greater than soil C:N (12.8±0.5) indicating that some immobilization of NH_4^+ may have occurred at those temperatures; however, in Pendleton Tilled soil the C:N of accumulated CO_2-C:inorganic N was never significantly greater than the soil C:N.

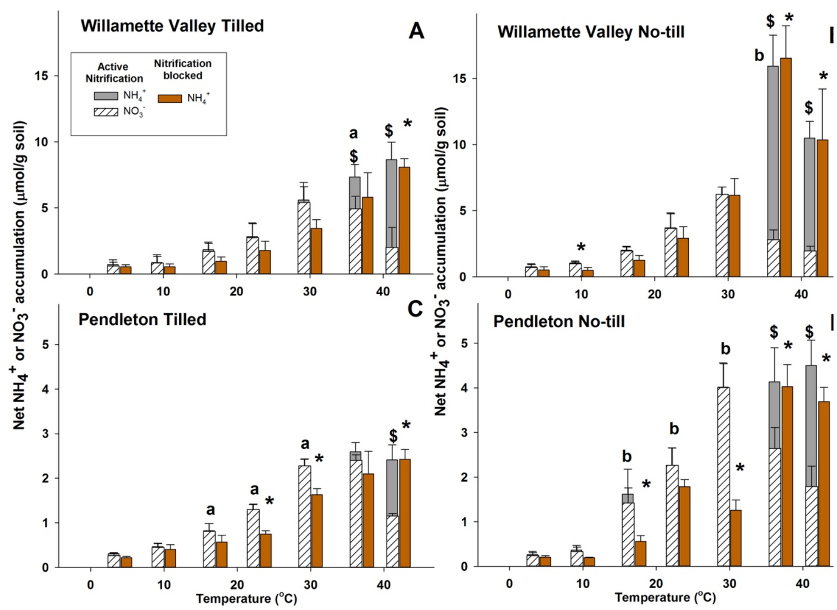

Figure 3. Inorganic N mineralization response across temperatures. The accumulation of net NO_3^- and/or NH_4^+ over 28 d in Willamette and Pendleton soils under Till and No-till management. Striped bars represent the net accumulation of NO_3^- over 28d in Willamette and Pendleton soils under Till and No-till management, and the gray bars represent the net NH_4^+ accumulated during active nitrification; the sum of the striped and gray bars represent the total mineralized NO_3^- + NH_4^+ during active nitrification. The orange bars represent the net NH_4^+ which accumulated when nitrification was blocked with low concentrations (10 μM C_{aq}) of acetylene. Data are the average of three field replicates and the error bars represent the standard deviation. ANOVA analysis indicated there were significant differences in NH_4^+ or NO_3^- over the temperature range; the statistics are not included here for readability. Asterisks (*) indicate a significant difference between NH_4^+ accumulated in the plus acetylene treatments and NO_3^- during active nitrification ($p \leq 0.05$). Significant NH_4^+ accumulations during active nitrification are indicated by '$' ($p \leq 0.05$). Lower case letters indicate temperatures at which NO_3^- accumulations in Till and No-till soil within a sampling site differ significantly ($p \leq 0.05$).

Willamette soils had higher concentrations of Total N than Pendleton soils, and there were greater accumulations of NO_3^- + NH_4^+ in soils of the same management type at multiple temperatures (Figure 2). The accumulation of NO_3^- + NH_4^+ in Willamette Tilled soil exceeded that of Pendleton Tilled at 17, 30, 37 and 42°C; and Willamette No-till soil exceeded that of Pendleton No-till at 4, 10, 30, 37 and 42°C ($p \leq 0.05$).

We found that active nitrification impacts the amount and speciation of N mineralized from SOM. We compared the net NH_4^+ which accumulated in treatments containing acetylene (nitrification blocked) to the total net NO_3^- + NH_4^+ that accumulated when nitrification was active (Figure 3). This comparison revealed that at some temperatures ≤ 30°C in all four soils that there was a trend for more NO_2^- + NO_3^- to accumulate during active nitrification than NH_4^+ released from SOM when nitrification was blocked. NO_2^- + NO_3^- was significantly greater than NH_4^+ (plus acetylene) at 10°C in Willamette No-till soil, at 23 and 30°C in Pendleton Tilled soil, and 17 and 30°C in Pendleton No-till soil ($p \leq 0.05$). This may indicate that at temperatures ≤ 30°C, that NH_3 oxidizers have the ability to outcompete heterotrophs for NH_4^+ released from SOM during respiratory processes, and that heterotrophic microorganisms were N limited in the presence of active nitrification. This trend seems to be more pronounced in Pendleton soils that have less Total C and N than Willamette soils of the same management type. However, at 37 and 42°C where biosynthetic processes are disfavored, there were no significant differences between accumulations of NO_3^- + NH_4^+ (active nitrification) and NH_4^+ (plus acetylene) in any of the soils (Figure 2). In Pendleton soils, NH_4^+ (plus acetylene) accumulated to the same extent at 37 and 42°C; while in Willamette Tilled soil significantly more NH_4^+ (plus acetylene) accumulated at 42°C than at 37°C, but the opposite was true in Willamette No-till soil ($p \leq 0.05$).

Willamette soils had higher concentrations of Total N (0.19% and 0.29% total N in Tilled and No-till soil, respectively), than Pendleton soils (0.09% and 0.15% total N in Tilled and No-till soil, respectively), and there were greater accumulations of NO_3^- + NH_4^+ in soils of the same management type at multiple temperatures (Figure 1). The accumulation of NO_3^- + NH_4^+ in Willamette Tilled soil exceeded that of Pendleton Tilled at 17, 30, 37 and 42°C; and Willamette No-till soil exceeded that of Pendleton No-till at 4, 10, 30, 37 and 42°C ($p \leq 0.05$).

BENEFITS & IMPACT: The assumption is generally made that soil microbes are C rather than N limited, and the mining of soil organic matter for C to fuel respiration results in NH_4^+ release (N mineralization) in excess of that needed for microbial growth. The activity of ammonia oxidizing bacteria and archaea in turn is limited by the rate of N mineralization, affecting the composition of inorganic soil N supporting plant growth. Despite the interconnected nature of respiration, N mineralization, and nitrification, there has been no comprehensive study of the response of these processes to the full range of environmental temperatures. In this study we observed significant thermodynamic differences between the processes in all four evaluated soils. The extent of these differences lead to distinctly different thermodynamic profiles in agricultural soils from eastern and western Oregon; and emphasizes the need to explore the relationship between these processes in other soils. Field or site specific estimates of basic soil processes will support modeling efforts to evaluate the potential for retention of N in soil organic matter, predict N mineralization to support crop yields, and reduce surplus N fertilization.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM: Anne Taylor has retired and will not seek any additional funding. Frank Chaplen continues to be interested in modeling of microbial processes and may build upon this work for future funding.

FUTURE FUNDING POSSIBILITIES: The data collected, and the model developed during this study will be used to justify funding from USDA/NIFA or NSF for a more comprehensive study that includes other variables that influence soil N mineralization in a broader range of environments. An interdisciplinary approach such as this proposal is of interest to funding agencies for studies that examine the effects of climate uncertainty on agricultural production and sustainability.