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Title: Determining nitrification capacity of Georgia blueberry soils

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Public Abstract

Information on potential nitrification in blueberry cultivation soils of Georgia are not currently available. Such information is useful in providing plants with the right form of nitrogen (N), thereby allowing for fine-tuning blueberry fertilization programs. The goal of this study was to determine the nitrification capacity of blueberry cultivation soils in Georgia. Soil samples were collected from six distinct sites across the blueberry cultivation regions of South Georgia. These sites had current southern highbush blueberry cultivation either with the cultivar ‘Farthing’ or ‘Optimus’. The samples were characterized for soil texture and various initial soil parameters such as nutrient concentrations. Further, the samples were incubated with or without the addition of N fertilizer. Subsamples were extracted from these in 1 M KCl to determine NH_4^+ and NO_3^- concentrations. These data will be used to determine nitrification capacities. The soil samples were classified either as Sand or Sandy loam soils. There was substantial variation in the initial pH, organic matter concentration, and carbon concentration, and the concentrations of nitrogen, phosphorus and potassium. Evaluation of NH_4^+ and NO_3^- concentrations from incubated samples to determine nitrification rates in currently underway in the PI and Co-PI’s labs.

Objectives: The main objective of the proposed research is:
To determine potential nitrification in blueberry cultivation soils in Georgia

Introduction

Blueberry (*Vaccinium* species) is a major fruit crop in the southeastern US, particularly in Georgia (GA). In Georgia, blueberry was cultivated on over 25,000 acres with a farmgate value over $348 million in 2020 (2021 Georgia Farm Gate Value Report). Sustaining the profitability of this industry requires optimization of production practices such as nutrient management. This is particularly true for nitrogen (N) nutrition. Nitrogen is a macronutrient which constitutes between 1.76 and 2% of the dry weight of healthy blueberry leaf tissue (Bryla and Strik, 2015; Doyle et al., 2021). Nitrogen deficiency during production can lead to stunted plant growth, and leaf chlorosis which is particularly evident in older leaves (Hart et al., 2006). *Vaccinium* sp. can acquire N as amino acids and peptides (organic forms), or in the inorganic forms. The two inorganic forms of N that are usable by plants are NH_4^+ (ammonium) and NO_3^- (nitrate). Blueberry plants, like many other Ericaceae plants, display two specific features that influence N nutrient management: 1. They are thought to display a preference for the NH_4^+-form over the NO_3^- form of N. Hence, NH_4^+-N based fertilizers are often used during blueberry cultivation. 2. Many cultivated blueberry types require low pH (acidic soils) for optimum growth. This requires that cultivation soils which generally display higher pH be amended to achieve the desirable pH of around 4 to 5.5.

Several previous studies suggest that blueberry plants display preference for the NH_4^+-form of N, while others indicate that they can use both forms of inorganic N (Cain, 1952; Oertli, 1963; Claussen and Lenz, 1999; Poonnachit and Darnell, 2004; Doyle et al., 2021). Preference for one form of inorganic N over another can occur either at the level of N acquisition from the media (soil), its translocation within the plant, or its utilization (Britto and Kronzucker, 2013; Alt et al., 2017). Recently, through research supported by the SRSFC, the PI's research demonstrated that blueberry plants display greater capacity for NH_4^+ uptake than they do for NO_3^- uptake, specifically when the available N concentration in the media is low or moderate (less than 0.25 mM). These results support previous studies where a preference for the NH_4^+ form of N has been suggested. Further, these results indicate that such preference is established at the level of N acquisition. However, these studies by the PI also indicated that at high N availability, blueberry plants display significant capacity for NO_3^- uptake. Hence, under cultivation conditions, if NO_3^- is abundantly available to blueberry plants, it is possible that they can acquire this form of N from the soil.

Ammonium is thought to be the predominant N-form available under low pH conditions while NO_3^- is the major available N-form under higher pH conditions. As blueberry cultivation involves soil amendment to low pH, and as NH_4^+-based N fertilizers are typically applied in blueberry production, it may be expected that the predominantly available N-source is the NH_4^+ form of N. However, NH_4^+ in soils can be converted to NO_3^- by soil-based microorganisms in a process referred to as nitrification. Ammonium is oxidized in the first step to hydroxylamine and then to NO_2^- (nitrite). These reactions can be performed by ammonia-oxidizing bacteria (AOB) such as *Nitrosomonas* sp. and *Nitrosococcus* sp. Nitrite is subsequently oxidized to nitrate by nitrite-oxidizing bacteria (NOB) such as *Nitrobacter* sp. At low pH (generally < 6.5) both these types of bacteria display limited growth and activity. Hence, until recently it was assumed that limited nitrification occurred in low pH soils such as those used for blueberry cultivation (Hu et al., 2014). Therefore, it was expected that the predominant N-form under low pH is NH_4^+ and that applied NH_4^+-based N fertilizers remain in the same form. However, recent studies have demonstrated that Archaea are capable of ammonia oxidation (Ammonia Oxidizing Archaea - AOA), and that they are numerically more abundant than AOBs in most soils. Therefore, AOAs are among the major contributors to ammonia oxidation (Konnecke et al., 2005; Leininger et al., 2006). Ecological studies have demonstrated that high nitrification rates are prevalent even in substantially acidic soils (Booth et al., 2005), and AOA have been isolated from acidic soils (Lehtovirta-Morley et al., 2011). Hence, AOAs likely play a predominant role in ammonia oxidation in acidic soils (Zhang et al., 2012). Further, presence of plants and long-term fertilization treatments can alter AOB and AOA population ratios such that AOAs play a dominant role in nitrification under acidic soil conditions (He et al., 2007; Chen et al., 2008).

In a previous study conducted outside the southeastern US (in Michigan), it has been demonstrated that nitrification occurs in low pH soils used for blueberry cultivation (Hanson et al., 2002). In that study, nitrification potential characterized at multiple sites indicated that blueberry cultivation led to greater nitrification capacity in comparison to neighboring forest soils. Further, higher nitrification in blueberry soils was associated with greater presence of nitrifying bacteria. This was attributed to repeated nutrition with NH_4^+-based N fertilizers. Similarly, Zebarth et al (2015) found that although nitrification capacity decreased with decreasing pH, substantial nitrification still occurred within a pH range of 4.5-5.2, a range typically recommended for blueberry cultivation. However, to our knowledge, such studies have not been performed in the southeastern US.

Together, these studies suggest that: 1. Nitrification can occur in acidic soils such as those used for blueberry cultivation; 2. Both forms of inorganic N (NH_4^+ and NO_3^-) are potentially present in blueberry cultivation soils, even under low pH and NH_4^+-N supply conditions. Hence, nitrification in blueberry cultivation soils can convert the available N-form into a source that is not as effectively acquired by blueberry plants, unless available at a high concentration. Further, nitrification can lead to leaching-related N losses which can limit the total N available to blueberry plants. Information on the nitrification potential at blueberry cultivation sites in the southeastern US, and particularly Georgia, is currently lacking. Hence, the aim of the current proposal is to determine potential nitrification capacity of blueberry cultivation soils in Georgia.

Materials and Methods

Blueberry cultivation soils from across southern Georgia were used for this study. A total of six farms (A-F) across the southern part of GA were sampled during mid-September 2024 (Table 1). These sites represented the major blueberry growing regions of south GA and included Pearson, Hoboken, and Alma regions. Blueberry cultivation at most of these sites has been ongoing for over five years. All six sites used in the study have southern highbush blueberry cultivation. While four of the sampled sites have ‘Farthing’ plantings, the other two have ‘Optimus’ blueberry. At each site, six-inch deep soil samples were collected from multiple rows using a hand-held shovel. Samples from four locations per site were pooled into a plastic container, labeled and transported to Athens, GA. The soil samples were homogenized, sieved through a 4 mm sieve and stored at room temperature. During storage, the samples were regularly aerated by hand mixing.

Multiple initial analyses were performed on the soil samples. The field moisture content was determined by weighing, drying and reweighing the soil samples. The water holding capacity was determined by adding water to saturation, allowing it to drain and re-weighing (Table 1). Soil texture analyses were performed at the AESL (in duplicate). Initial soil characteristics were also analyzed at the AESL at UGA (in triplicate). The parameters analyzed were, pH, lime buffering capacity (LBC), soil nutrient analysis, organic matter concentration, and percent carbon (C) and N.

Nitrification capacity of the soils is being determined currently. For this objective, the soil samples from each site were re-wet to 50% water holding capacity. Sub samples (300 g) from each site were transferred into 960 mL glass jars. Two treatments were applied to the soil samples. 1. Control: no additional of N; 2. N Fertilized: Ammonium fertilizer $[NH_4)_2SO_4]$ was added at the rate of 12.7 pounds per acre. Four replicates per soil sample per treatment were used. The jars were incubated at room temperature. Soil samples were collected from the jars at regular intervals (in progress, until ~100 d after initiation). The samples were extracted in 1M KCl by shaking at room temperature for 30 min. The extractants were allowed to settle, and the supernatant was decanted and stored at -20 °C. These extractants will be analyzed using the Timberline Ammonia Analyzer to determine NH_4^+ and NO_3^- concentrations.

Sample	Location (GA)	Cultivar grown (southern highbush blueberry)	Initial moisture content (g g^{-1})	Water holding capacity (100%; g g^{-1})
A	Pearson	Farthing	0.11	0.26
B	Hoboken	Farthing	0.07	0.39
C	Hoboken	Optimus	0.12	0.27
D	Alma	Farthing	0.12	0.33
E	Alma	Optimus	0.10	0.27
F	Alma	Farthing	0.14	0.29

Results and Discussion

The soil samples collected from south GA were largely classified as Sandy soils or Sandy loam soils (Table 2). The soils displayed an acidic pH with a range of 4.17 (E) to 5.29 (C) (Table 3). Initial organic matter in the soils ranged from around 1.2 % (C) to around 3.5% (D). The initial carbon concentration of the soils ranged from 0.6 % (C) to 2.1 % (D), while N concentration varied by about 3-fold from 0.033 % (C) to 0.097 % (F).

Table 2. Soil texture analysis.

Sample	Sand (%)	Silt (%)	Clay (%)	Soil type
A	88.7	7.34	3.96	Sand
B	90.5	7.77	1.73	Sand
C	93.95	3.64	2.41	Sand

D	89.32	8.49	2.19	Sand
E	87.37	8.61	4.02	Sand
F	73.37	12.42	14.215	Sandy loam

Table 3. Initial soil analyses.

Sample	pH	Organic matter (%)	Carbon (%)	Nitrogen (%)	Phosphorus (mg/kg)	Potassium (mg/kg)
A	5.09 ± 0.2	1.87 ± 0.3	1.07 ± 0.3	0.07 ± 0.01	98.6 ± 9.0	35.3 ± 1.9
B	5.20 ± 0.1	3.42 ± 0.3	2.06 ± 0.4	0.09 ± 0.02	33.6 ± 1.9	85.0 ± 5.2
C	5.29 ± 0.1	1.23 ± 0.3	0.58 ± 0.2	0.03 ± 0.01	57.8 ± 7.3	15.5 ± 0.7
D	4.18 ± 0.1	3.48 ± 0.1	2.13 ± 0.3	0.07 ± 0.01	7.68 ± 0.7	36.4 ± 1.0
E	4.17 ± 0.0	2.31 ± 0.0	1.47 ± 0.1	0.06 ± 0.01	9.07 ± 2.2	36.1 ± 7.0
F	4.29 ± 0.1	2.96 ± 0.6	1.83 ± 0.3	0.10 ± 0.01	57.0 ± 6.0	65.1 ± 4.4

Analyses of NH_4^+ and NO_3^- from extracted samples at various stages of incubation is currently in progress. These data will be presented as they become available in early 2025.

Significance:

This study will help determine potential nitrification in blueberry cultivation soils. Such information will be useful in determining the forms of N available to plants, and to fine-tune nutrient management strategies in blueberry production. For example, if substantial nitrification is noted in southern Georgia blueberry cultivation soils, application of nitrification inhibitors may prove a valuable approach to mitigate N losses, and to provide N to plants in a more usable form.

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