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Soil moisture gradients shape microbial communities and influence cranberry yield: a case study on subirrigation

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Water management is vital in cranberry farming, balancing plant needs and supporting flood-based harvesting. This study examines subirrigation, a technique that reduces water use and enhances yields, by utilizing natural soil moisture gradients in two fields to assess its effects on yield and soil bacteria. Considering 166,551 observation points collected over four years, we confirmed significant differences in soil moisture between the eastern and western sides of the fields, with lower water table depths on the subirrigated sides. Using 16 S rRNA sequencing, we examined soil bacterial communities, focusing on nitrogen cycling. Subirrigated areas, with lower moisture levels, showed higher cranberry yields (up to 45.67 t/ha) and a greater abundance of beneficial bacteria such as *Burkholderia* and *Arthrobacter*. The results also suggest an increase in predicted bacterial genes linked to nitrogen mineralization, denitrification, and nitrate assimilation as soil moisture levels rise, which, notably, correlates negatively with cranberry yield. Conversely, DNRA (*nirD*) and ANRA (*NasA* and *NasB*) genes appear to be indirectly favored in environments with lower soil moisture. Our findings not only shed light on the intricate relationships between bacterial genera, nitrogen metabolism, and environmental factors but also underscore the potential of sustainable agricultural practices in enhancing soil health.

Keywords *16S rRNA sequencing*, *Cranberry yield*, *Soil water*, *DNRA*, *Subirrigation*, *Water management*

Cranberry (*Vaccinium macrocarpon*) is a native North American fruit known for its potential health benefits, including the prevention of oral diseases[1]urinary tract infections[2]and the enhancement of immune system function[3]. Cranberries are cultivated in artificial swamps, with their growth sensitive to variations in soil moisture, which can impact crop yield[4]. Previous studies have indicated that controlling the water table to 600 mm below soil surface results in the optimal conditions for cranberry production[4]. This water management strategy, obtained by controlled drainage or subirrigation[5]may decrease water irrigation use by 77% and improve cranberry yield by 79%[6].

Cranberry plants have nitrogen conservation strategies, absorbing only ammonium in the soil and producing organic material with a high C/N ratio, reducing the decomposition rate[7]. These characteristics enhance nitrogen acquisition from the soil in an environment that predominantly becomes anaerobic more than three times a year, such as for pest control (spring and fall) and frost protection during the winter[8,9]. Denitrification is a critical microbial process in the nitrogen cycle, responsible for the conversion of nitrate (NO_3^-) and nitrite (NO_2^-) into nitrogen gas (N_2) or nitrous oxide (N_2O), thereby facilitating the removal of excess nitrogen from ecosystems, but contributing to greenhouse gas (GHG) emissions. In fact, it has been shown that increasing soil moisture can induce nitrogen losses through denitrification process[10]. This process is predominantly carried out by denitrifying bacteria, which utilize nitrate as an alternative electron acceptor during anaerobic respiration when oxygen is limited[11].

Meanwhile, it was observed that cranberry fields established on former peat extraction sites exhibit lower GHG emissions compared to active peat extraction sites and undisturbed raised bogs[12]. The authors estimated that annual N_2O emissions from the cranberry fields could reach 0.18 ± 0.15 kg ha^{-1} $year^{-1}$. Currently, the role of the soil microbial community in the biogeochemical maintenance of cranberry-cultivated soils remains

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largely unknown. However, Stackpoole et al. (2008)[7] suggested that cranberry establishes a symbiosis with soil microorganisms, including ericoid mycorrhizal fungi and other microbes that may be involved in the nitrogen and carbon cycles, thereby facilitating its nutrient uptake. Thus, as the microbial community of cranberry soils remains poorly studied, it is essential to continue our research to elucidate its role in biogeochemical dynamics, assess its influence on soil fertility, and better understand its impact on nutrient cycling.

Nitrate reduction to ammonium (NRA) serves as a crucial pathway for nitrogen conservation in soil, mitigating nitrogen losses via nitrate leaching and N_2O gas emissions, as detailed by Pandey et al. (2020)[13]. The NRA process encompasses two primary steps: the initial reduction of nitrate (NO_3^-) to nitrite (NO_2^-), followed by its further reduction to ammonium (NH_4^+). Depending on the subsequent utilization of the produced ammonium, this process can be categorized into two types: assimilatory nitrate reduction to ammonium (ANRA), where ammonium is assimilated, and dissimilatory nitrate reduction to ammonium (DNRA), where it remains unassimilated by soil microorganisms. Cheng et al. (2022)[14] identified through a meta-analysis that precipitation, temperature, pH, soil total carbon and nitrogen are the main regulators of DNRA in soil. Bacterial DNRA/ANRA may then help cranberry plants to conserve nitrogen in the soil. Thus, the NRA process has the potential to reduce nitrogen losses, subsequently decreasing environmental impact, and increasing availability for cranberries (which exclusively absorb ammonium). However, the bacterial community in cranberry soils, especially the functional groups associated with the NRA process, remains unexplored.

Soil moisture significantly affects the microbial community by affecting microbial activity, diversity and community composition. It is widely acknowledged as a key factor influencing prokaryotic richness and diversity in soil[15–17]. Soil moisture levels below a critical threshold can have a notable negative impact on microbial functions, such as reducing respiration and decomposition rates. Different microbial taxa respond variably to humidity levels. For example, specific microbial groups such as *Acidobacteria* and *Verrucomicrobia* exhibit differential abundance responses to water stress, highlighting the sensitivity of microbial communities to variation in soil moisture, particularly in sandy soil[15]. Similarly, high water content decreases oxygen content, favoring anaerobic microbes over aerobic microbes. This change can significantly alter the diversity and richness of soil microbial communities. Thus, soil water content significantly influences the structure and function of microbial communities, thereby affecting key ecological processes such as carbon cycling and biogeochemical cycling of soil nutrients, particularly nitrogen[18].

In this study, we specifically selected two cranberry fields, labeled L1 and L3, due to their inherent soil moisture variations between the eastern (E) and western (W) sides. This unique setup allowed us to comprehensively investigate the pre-harvest soil bacterial communities across four distinct sides (L1W, L1E, L3W, and L3E). Our objectives were: *(i)* to identify the bacterial community in cranberry soils under conventional irrigation and subirrigation; *(ii)* to evaluate the occurrence of DNRA/ANRA predicted genes in these soils; and *(iii)* to examine their correlation with soil moisture and cranberry yield.

Results

Our dataset was composed of 166,551 observation points (from 2017 to 2020) on water table depth that enabled the historical difference in soil moisture between the eastern (E) and western (W) sides of the two cranberry fields (L1 and L3). The results indicated a significant difference between water table depth of the East (395.8 mm of average dpth) and West sides (*600* mm of average depth). Boxplot and ANOVA test (considering $p\text{-value} < 0.05$) are presented in Supplementary Figure S1.

Soil moisture, soil chemistry, and cranberry yield

Shallow (0–25 cm) soil samples were collected from monitored cranberry fields (L1 and L3) along transects on their W and E sides, perpendicular to the subsurface drain system (Fig. 1). Soil chemical properties were determined by the total (*tot*) and available (*av*) soil elements (*see Material and Methods*). Principal component analysis (PCA) showed pH, soil moisture, $Rb_{(tot)}$, $Sr_{(tot)}$, $P_{(av)}$, $Mg_{(av)}$, $Fe_{(tot)}$, $K_{(tot)}$, $Ca_{(tot)}$, and $Zn_{(tot)}$ as main components across Dimension 1 and 2 (Supplementary Figure S2). For the soil moisture, the field and regions showed a significant difference between the W and E sides of the L1 field (ANOVA, $p\text{-value} < 0.05$), while the L3 field sides showed no difference (Fig. 2A).

Cranberry yield was quantified and interpolated by kriging method for each sampling point (Supplementary Figures S3 and S4). The L1 field showed the highest (W side, 45.67 Mg/ha) and lowest (E side, 26.15 Mg/ha) average cranberry yields, while there was no difference in cranberry yield between the two L3 field sides (Fig. 2B). Pearson's correlation was performed to identify the soil moisture and soil chemistry factors that presented a significant correlation with cranberry yield. Based on Pearson correlation coefficient (r), cranberry yield shows positive correlations with $Cu_{(av)}$ ($r = 0.48$, $p\text{-value} < 0.001$), and negative correlations with soil moisture ($r = -0.56$, $p\text{-value} < 0.001$) and $Mg_{(av)}$ ($r = -0.24$, $p\text{-value} < 0.05$) (Figure S5).

Soil bacterial taxonomic composition and redundancy analysis with chemical factors

A total of 11,273 amplicon sequence variants (ASVs) were assigned to the domain Bacteria. Phyla *Proteobacteria* (32.65%), *Actinobacteria* (30.60%), and *Acidobacteria* (12.43%) accounted for 75% of the relative abundance, considering all soil samples (Fig. 3A). Figure 3B shows the relative abundance at phyla level for each Field (L1 and L3) and Side (E and W), which were compared by Kruskal-Wallis rank-sum test (considering $p\text{-value} < 0.05$). The results revealed a significant variation of the five most abundant phyla in the L1 field between the E and W sides. For the L1 field, Fig. 3B shows a high abundance of the phyla *Proteobacteria*, *Actinobacteria*, and a low abundance of the phyla *Acidobacteria*, *Chroloflexi* and *Firmicutes* in the W side. In the L3 field, the difference between the phyla followed a similar pattern, with greater abundance of phyla *Acidobacteria* and *Chroloflexi* in the E side. Interestingly, the L1 W side had the highest relative abundance of the phylum *Proteobacteria* (relative abundance of 40%), and the L1 E side had the lowest abundance of the *Actinobacteria* phylum (23.31%).

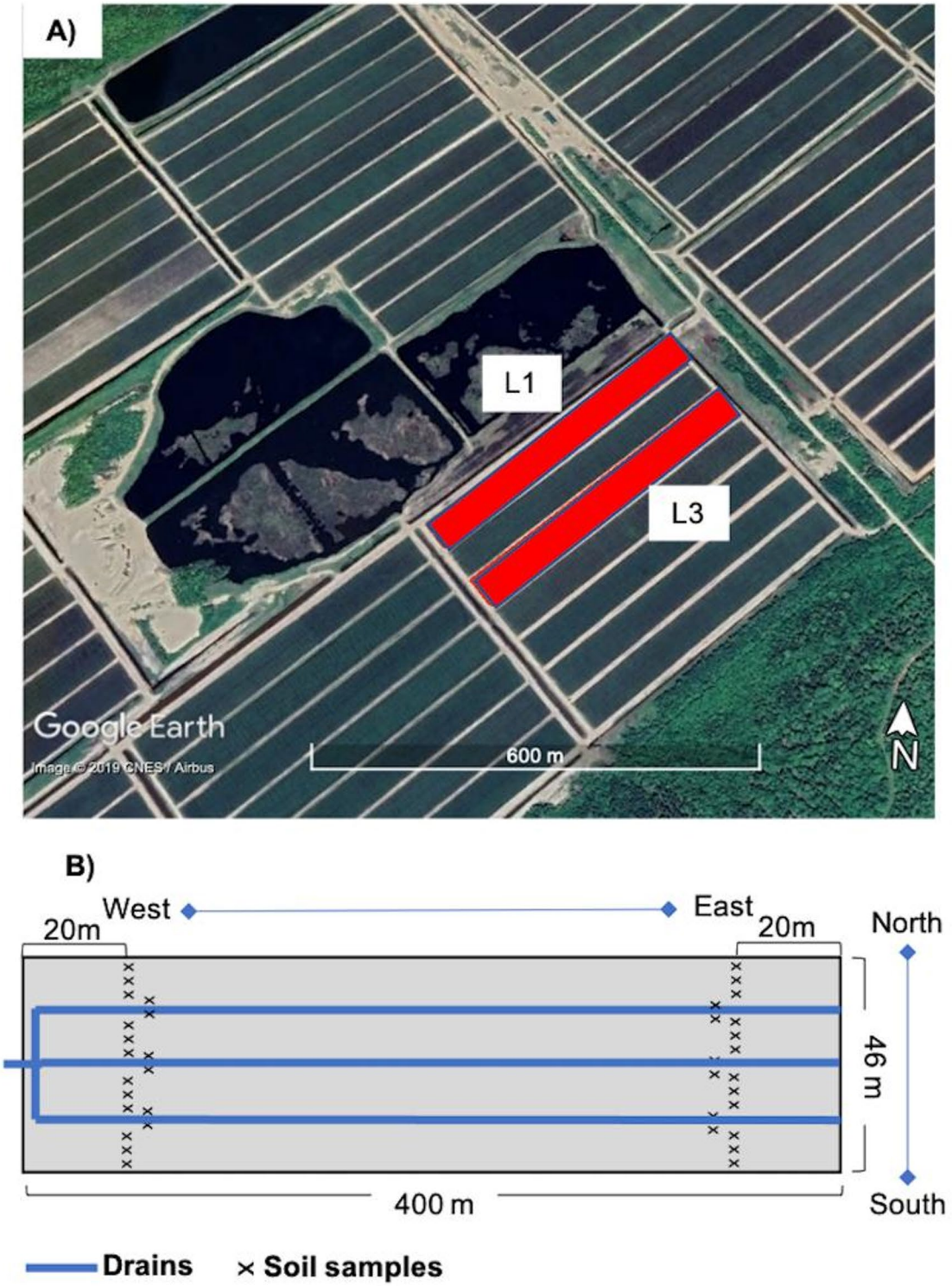

Fig. 1. **A**) Map of the experimental area showing the cranberry production fields L1 and L3 used in the study; **B**) Soil sampling design to assess the effects of sides (East and West) and locations between drainage lines within each field.

The differential abundance analysis using ANCOM-BC2 identified several bacterial genera with statistically significant variation across field sides and locations (Fig. 4). L1 East was used as the reference group, so positive $\log_2$ fold change (lfc) values indicate higher abundance in the comparison group relative to L1 East, while negative values indicate lower abundance. The most prominent genus was *Burkholderia-Caballeronia-Paraburkholderia*, with lfc = 4.40 in L1 West and lfc = 3.53 in L3 West, indicating a marked enrichment in the subirrigated zones.

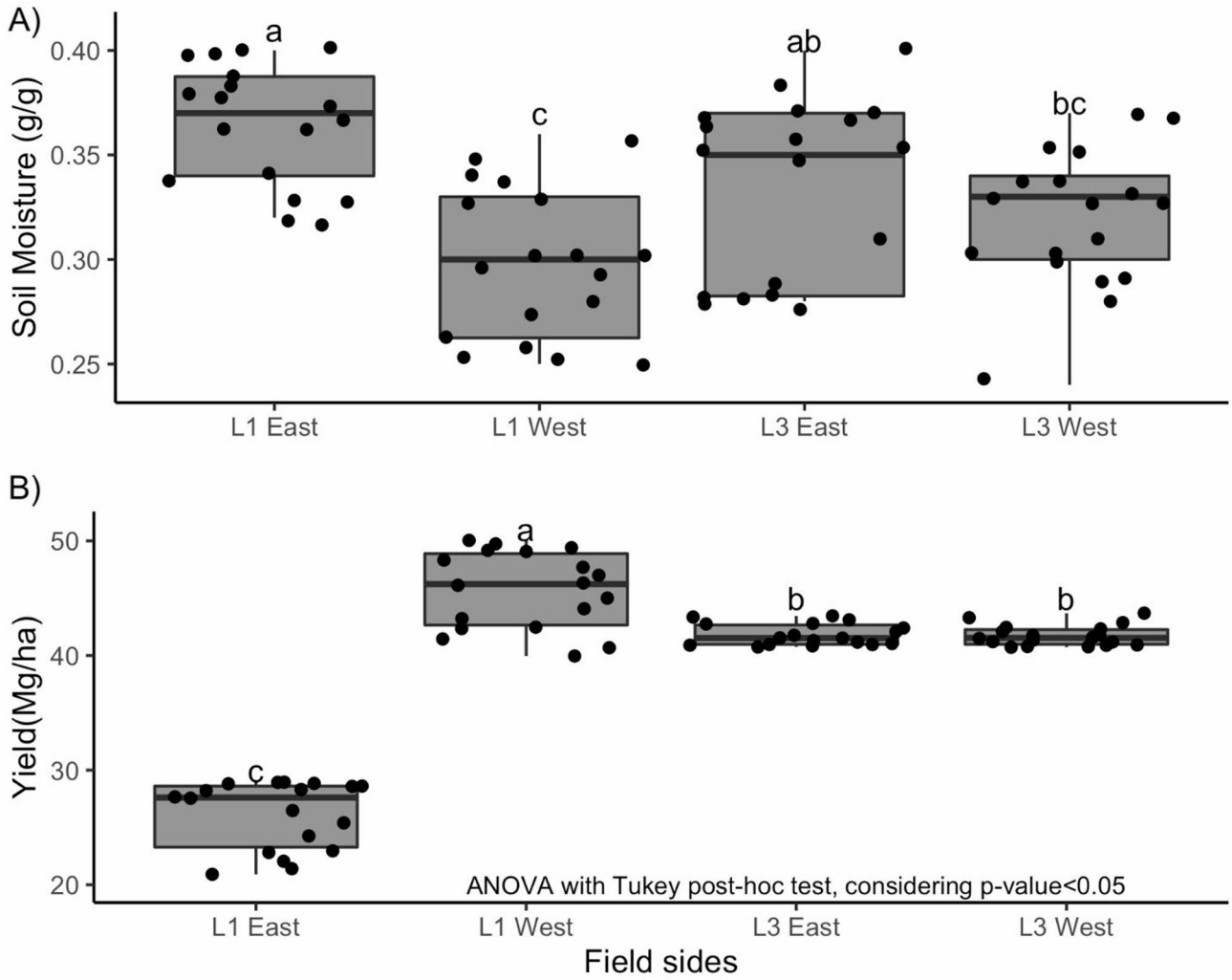

Fig. 2. **A**) Soil moisture content (g/g) in each cranberry side by field (L1 West, L1 East, L3 West, and L3 East); **B**) Cranberry yield (Mg/ha) in each cranberry side by field (L1 West, L1 East, L3 West, and L3 East). Averages were compared using ANOVA and ranked by Tukey's test (*p-value < 0.05*).

Similarly, *Paenibacillus* showed lfc = 2.68 in L1 West and lfc = 2.12 in L3 West, while *Pseudarthrobacter* reached lfc = 2.92 in L3 West. These values suggest a consistent increase in abundance of these genera in the West sides when compared to L1 East. Other genera followed this trend, including *Rhodanobacter* (lfc = 2.20 in L1 West, 1.83 in L3 West), *Massilia* (lfc = 3.83 in L3 East, 2.58 in L1 West, 2.02 in L3 West), and *Arthrobacter* (lfc = 2.05 in L1 West). These patterns reflect a strong and consistent microbial shift across the West and East sides of both fields.

Conversely, several genera showed higher relative abundance in L1 East, reflected by negative or near-zero lfc values in other groups. For example, *Bryobacter* (lfc = −0.96 in L1 West), *Candidatus Ovatusbacter* (lfc = −1.54 in L1 West), TM7a (lfc = 2.18 in L1 West but strongly negative in the intercept), and *Hymenobacter* (lfc = 1.97 in L3 East, but low elsewhere) exhibited opposite abundance patterns. *Sphingomonas* and *Actinospica* also showed modest fold changes, but were consistently more abundant in L1 East.

To assess the influence of environmental variables on soil bacterial communities, we performed a distance-based Redundancy Analysis (dbRDA) and a Redundancy Analysis (RDA) triplot. The dbRDA was based on Bray-Curtis dissimilarity and included only the environmental variables that were statistically significant in the PERMANOVA (adonis2) model. This ordination (Fig. 5A) explained 67.2% and 10.8% of the variance on the first two axes, respectively. The vectors indicate that Moisture, Yield, Mg.av, Ca.av, Mn.av, and Cu.tot were the main environmental drivers shaping the structure of the soil microbial communities.

These findings were supported by the PERMANOVA analysis (adonis2, 999 permutations), which confirmed that Moisture ($R^2 = 0.0468$, $p = 0.001$), Yield ($R^2 = 0.0406$, $p = 0.001$), Mg.av ($R^2 = 0.0387$, $p = 0.001$), Ca.av ($R^2 = 0.0381$, $p = 0.002$), Mn.av ($R^2 = 0.0352$, $p = 0.002$), and Cu.tot ($R^2 = 0.0246$, $p = 0.026$) were significantly associated with microbial variation (Supplementary Table 1). Variables that were not significant in the PERMANOVA were included in preliminary models but excluded from the final visualization to enhance clarity.

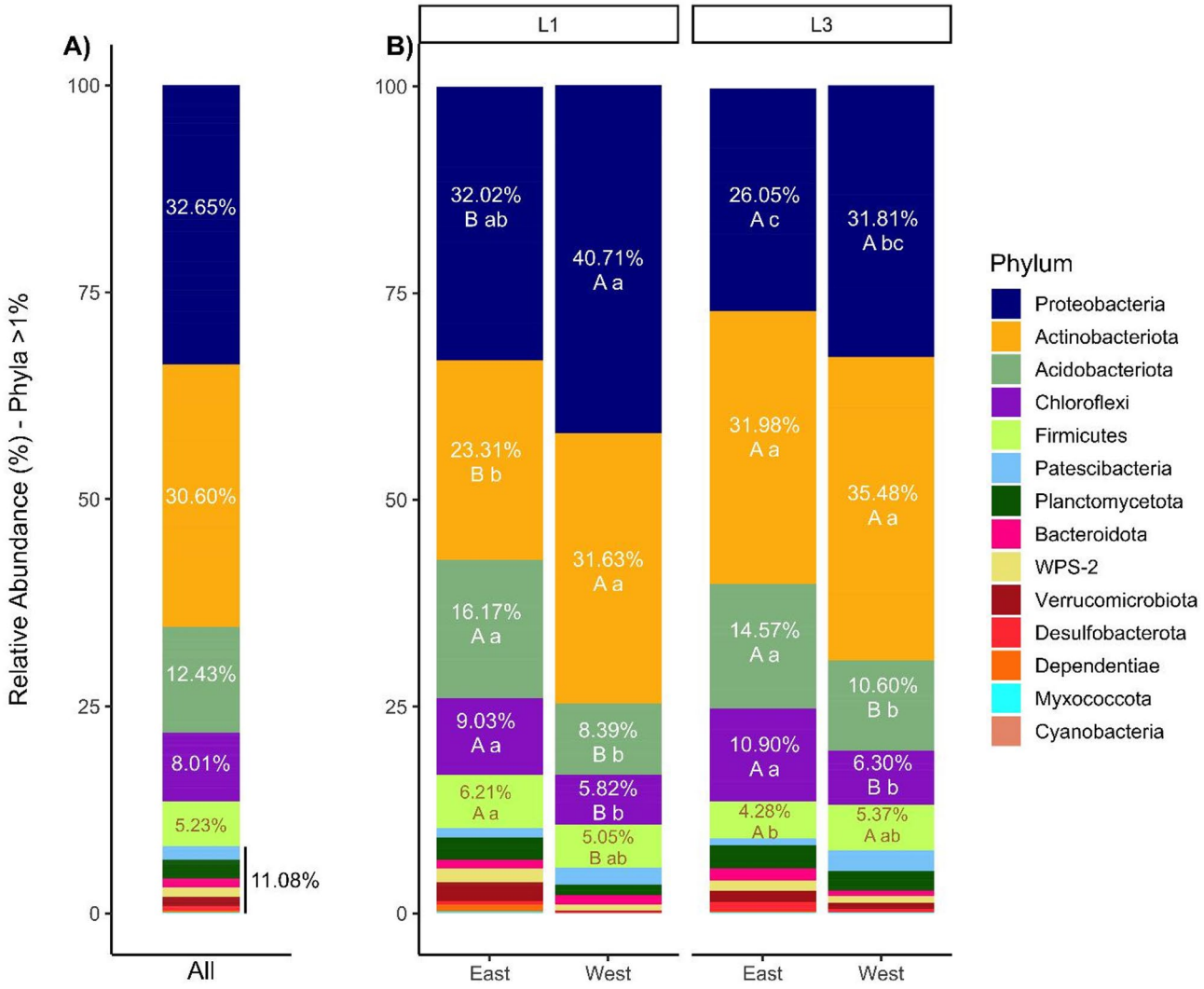

Fig. 3. **A**) Total relative abundance of soil bacterial communities across all soil samples; **B**) Average relative abundance of bacterial phyla in each cranberry side by field (L1 West, L1 East, L3 West, and L3 East). Averages were compared using the Kruskal-Wallis test followed by Dunn's post-hoc test (p-value < 0.05). Uppercase letters indicate comparisons between East and West sides of each field, considering each phylum. Lowercase letters indicate comparisons of phylum abundance between sides (L1 East, L1 West, L3 East, and L3 West).

To complement this community-level analysis, we performed an RDA triplot at the genus level (Fig. 5B), projecting key genera that responded to the same significant environmental variables. This analysis revealed distinct patterns of bacterial associations: genera such as *Burkholderia-Caballeronia-Paraburkholderia*, *Arthrobacter*, and *Rhodanobacter* were positively associated with the L1 West and L3 West sides, which correspond to the subirrigated zones with lower soil moisture. In contrast, genera such as *Bryobacter* and *Acidothermus* were more associated with the higher-moisture zones on the East sides of both fields. These observations provide insight into the potential microbial mechanisms underlying plant-soil feedbacks under long-term moisture gradients.

Predictive functionality of soil microbial community in cranberry soils

Functional prediction analysis unveiled a significant abundance of genes related to the nitrogen cycle (*see* Supplementary Figure S6). Figure 6 illustrates a complex network analysis highlighting the interactions between genes involved in nitrogen metabolism and environmental variables such as yield and moisture, including the values of Pearson's correlation r ($p\text{-value} < 0.05$). The first highlighted is the negative correlation between yield and moisture (r=−0.54), confirming the negative relationship between these two variables. Significantly, genes such as those linked to nitrogen mineralization (*hcp*; r=−0.62), denitrification (*NirS*; R=−0.57), and nitrate assimilation genes *NrtD* (r=−0.73) and *NrtB* (r=−0.58) all displayed negative correlations with yield. Conversely, *NrtB* (r = 0.54) and *NrtD* (r = 0.57) manifested positive correlations with soil moisture. A comprehensive list of Pearson's correlation (Pearson R) values for all interactions is provided in Supplementary Table S1.

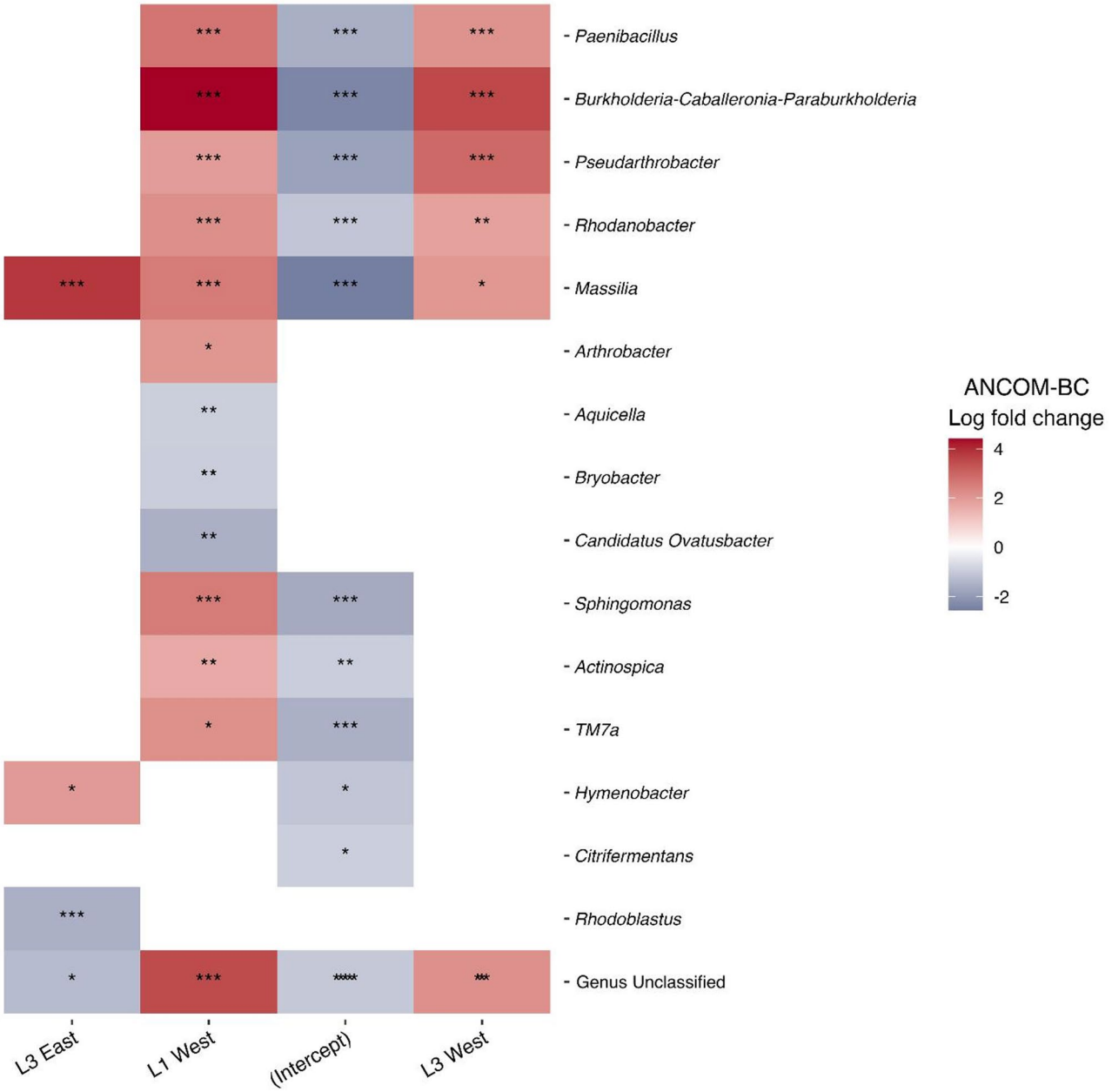

Fig. 4. Differential abundance of bacterial genera across field groups identified using ANCOM-BC2. The heatmap displays $\log_2$ fold changes in relative abundance for each genus across groups (L1 East[Intercept], L1 West, L3 East, and L3 West). Red indicates higher abundance, and blue indicates lower abundance. Asterisks represent levels of statistical significance ($p < 0.05$, $p < 0.01$, $p < 0.001$). Genera names are displayed on the Y-axis in italics, and "Genus Unclassified" refers to unidentified taxa at the genus level.

A noteworthy observation is the synergistic relationship between nitrogen fixation genes and those participating in denitrification, hydroxylamine reduction, and nitrate assimilation. For instance, *hcp* exhibits positive correlations with genes like *NirS*, *NorB*, *NorC*, *NrtB*, *NrtD*, *nifK*, and *nifH*. However, the *hcp* gene showed a higher correlation with nitrogen-fixing genes (r_{avg}=0.92) than genes associated with denitrification (r_{avg}=0.37) and nitrate assimilation genes (r_{avg}=0.42). Supplementary Figures S7–S11 detail the relative abundance variations of nitrogen cycle-related genes across different fields (L1 and L3) and sides (E and W). These findings bolster the insights presented in Fig. 5, suggesting that genes linked to nitrate assimilation, denitrification, nitrogen fixation, and mineralization (especially the *hcp* gene) were more abundant in humid soils. This indicates a direct correlation between soil moisture and microbial activity pertinent to the nitrogen cycle in cranberry soils, with humid sides promoting the expression of these specific genes.

The genes *NasA*, *NasB*, *NirA*, and *NarB* are central to the assimilatory nitrate reduction process, each displaying unique correlation dynamics. *NarB* and *NirA*, for instance, correlate positively with nitrate assimilation genes (*NrtB*, *NrtC*, *NrtD*) with Pearson's correlation r_{max}=0.81 and r_{min}=0.22, respectively, and with the denitrification

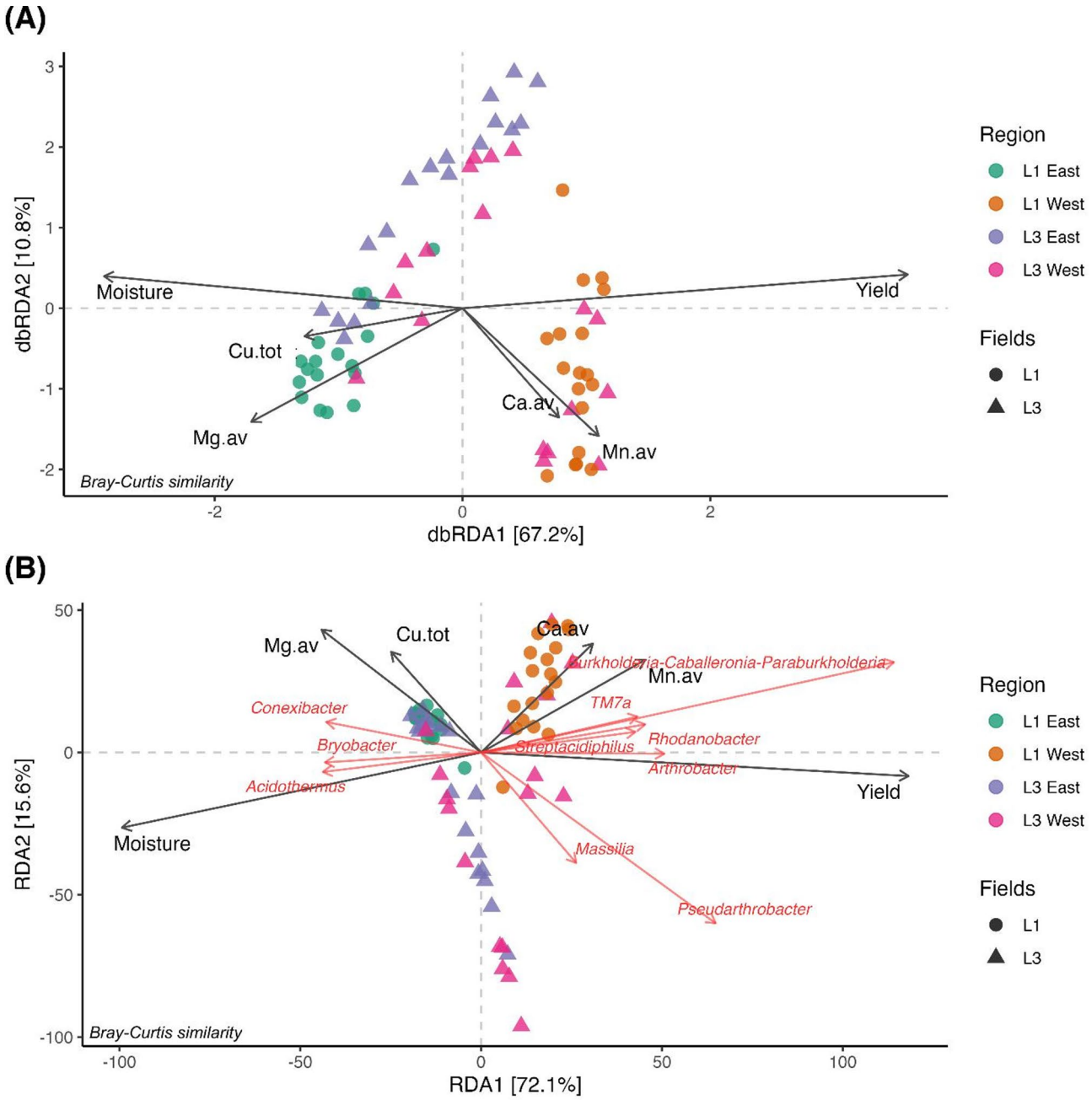

Fig. 5. Constrained ordination of bacterial communities and environmental variables, showing the relationship between bacterial community structure and selected environmental variables that were significant in the PERMANOVA model ($p < 0.05$). (A) Distance-based Redundancy Analysis (dbRDA). Arrows represent the direction and strength of the correlation between environmental variables and microbial composition. Sample points are colored by field side (East vs. West) and shaped by field identity (L1 vs. L3). (B) Redundancy Analysis (RDA) triplot at the genus level, displaying the correlation between selected soil bacterial genera (in red), significant environmental variables (in black), and the distribution of samples. The first two axes explain 72.1% and 15.6% of the total variance, respectively. Genera such as *Burkholderia-Caballeronia-Paraburkholderia*, *Arthrobacter*, and *Rhodanobacter* were associated with the West sides of the fields (L1W and L3W), corresponding to the subirrigated zones with lower soil moisture, while other genera were more frequent in the East (non-subirrigated) sides.

gene *NorC* ($r = 0.34$). This suggests their indirect enhancement by heightened soil moisture. In contrast, *NasA* and *NasB* exhibit correlations with the *NirD* gene (mean $r = 0.62$), associated with dissimilatory nitrate reduction, hinting at their indirect association with drier soils and, consequently, elevated yields. The multifaceted *NarG* gene, pivotal in the nitrogen cycle, deserves special attention. Engaged in diverse processes like dissimilatory

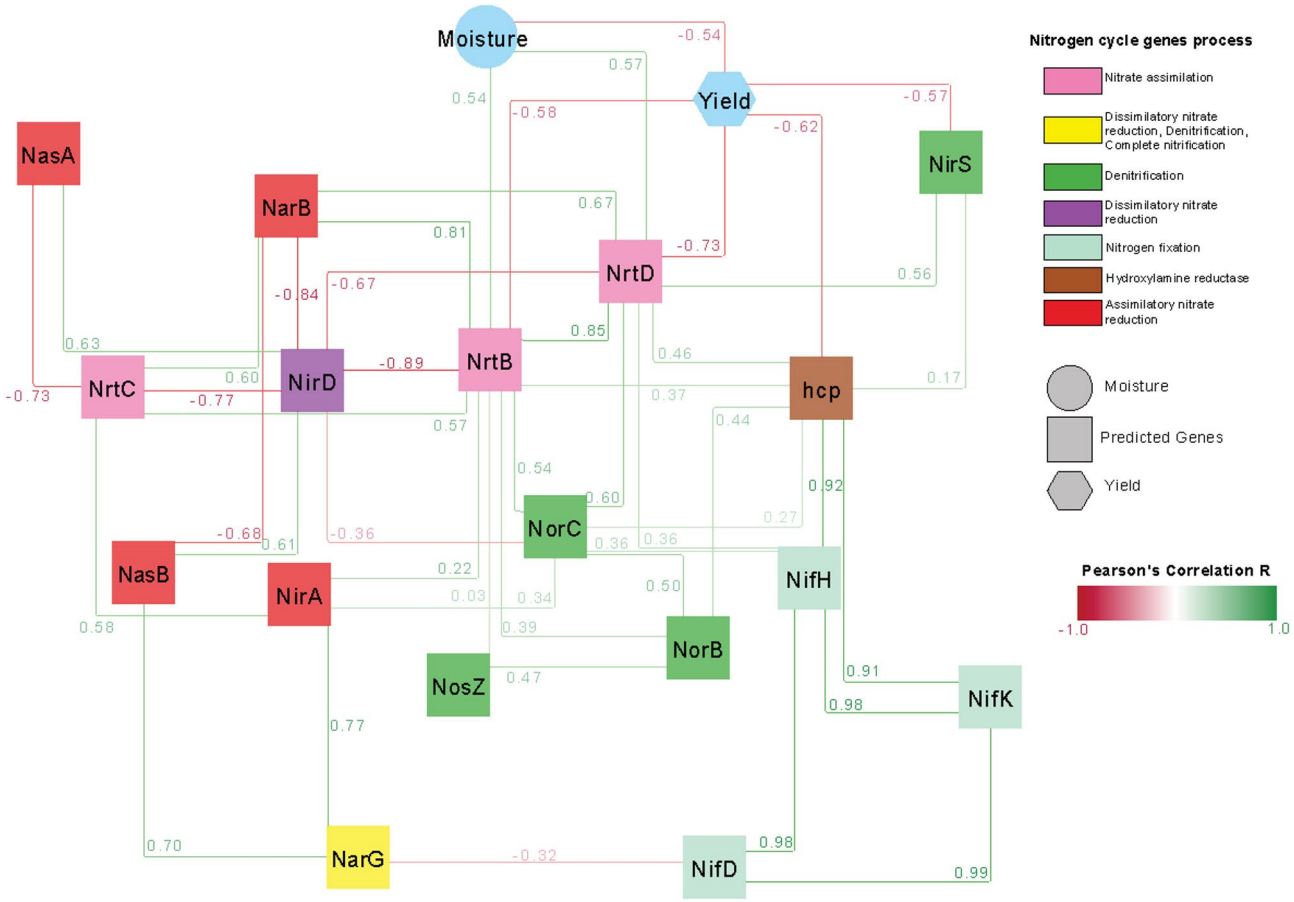

Fig. 6. Network analysis showing the associations between soil parameters (Moisture and Yield) and the predicted abundances of nitrogen cycle genes (represented by different symbols). The network includes several pathways, represented by colored edges: nitrate assimilation, dissimilatory nitrate reduction, denitrification, complete nitrification, hydroxylamine reductase, nitrogen fixation, and assimilatory nitrate reduction. Pearson's correlation coefficients (R) were used to establish significant correlations between the parameters and gene abundances ($p < 0.05$). The thickness and color of the edges indicate the strength and nature of the correlations, with thicker edges representing stronger significant correlations.

nitrate reduction, denitrification, and complete nitrification, network analysis indicates its positive correlations with *NirA* ($R = 0.77$) and *NasB* ($R = 0.70$) genes, both integral to assimilatory nitrate reduction.

Together, metabolic pathways encompassing nitrate assimilation, denitrification, fixation, and mineralization (facilitated by hydroxylamine reductase) generally exhibit a negative correlation with cranberry yield. Conversely, genes like *NirD*, *NasA*, and *NasB*, active in both assimilatory and dissimilatory nitrate reduction, seem to foster a positive, albeit indirect, relationship with cranberry yield.

Discussion

Our investigation of the bacterial community in cranberry soils aimed to unravel the complex interplay between soil bacterial dynamics and cranberry production, with an emphasis on functional groups linked to nitrogen metabolism, such as the DNRA process. We hypothesized that variations in soil moisture, especially differences between the E and W sides of the fields, would significantly influence the composition of the bacterial community and, consequently, cranberry yield. This hypothesis is supported by previous studies demonstrating the direct impact of soil moisture on nitrogen cycling and microbial activity[19]. Results confirmed a marked difference in bacterial community between these sides, with a strong negative correlation between soil moisture and cranberry productivity. We observed that the genes associated with the processes of DNRA (*nirD*) and ANRA (*NasA* and *NasB*) were more abundant in areas managed with subirrigation, suggesting that this practice may favor the conservation of nitrogen in the soil. This reinforces the importance of understanding microbial interactions to improve sustainable agricultural practices, as also seen in the study of Pandey et al. (2020)[13]which highlighted the role of DNRA in nitrogen retention and reducing leaching losses and greenhouse gas emissions.

The western part of the studied fields was managed through subirrigation, allowing for controlled water table depth (WTD) and reduced soil moisture. In contrast, the eastern part, with naturally higher WTD, experienced greater soil moisture. Our results confirmed the inverse relationship between soil moisture and cranberry yield: L1 W, with lower moisture, had the highest yield, while L1 E, with higher moisture, showed the lowest. This

pattern is consistent with the findings of Pelletier et al. (2015)[6] and Caron et al. (2016)[20]. Additionally, the reduced moisture on the western side may have favored bacterial communities involved in nitrogen retention processes, such as those participating in DNRA, which could have contributed to the higher yield.

A direct correlation between cranberry yield and copper (Cu), but an inverse correlation with magnesium (Mg) was observed. Copper, a crucial plant micronutrient, plays a vital role in enzymes responsible for processes such as photosynthesis, respiration, and seed formation[21]. Interestingly, soil moisture significantly influences copper concentrations, with drier conditions leading to higher copper levels[22]as shown by the db-RDA between the L1 W side and the Cu vector. Although magnesium is essential for chlorophyll production and enzyme activity regulation[23,24]it can also interfere with the uptake of other cations, such as calcium and potassium[25]. The presence of magnesium can influence the activity of specific microbial communities involved in nitrogen cycling, thus affecting the overall nitrogen balance within ecosystems[26]. Moreover, magnesium has a regulatory role in the transcription of genes linked to the nitrogen cycle, as observed in studies that demonstrate its influence on gene expression, such as those associated with nitrate reduction and nitrogen assimilation[27,28]. These findings suggest that the interaction between soil moisture and nutrient availability can have significant implications for both microbial activity and plant yield.

The bacterial community in cranberry soils plays a key role in maintaining crop health and productivity. While studies on the cranberry rhizosphere are still emerging, previous research has identified *Proteobacteria*, *Actinobacteria*, and *Acidobacteria* as dominant phyla in these soils[29–31]. Our findings are consistent with this trend, particularly highlighting the prevalence of these groups in both L1 and L3 field sites. Although functional traits such as nitrogen cycling cannot be broadly attributed to entire phyla, many of the dominant genera identified in our study—which belong to *Proteobacteria*— includes several genera that are involved in beneficial plant interactions and key nitrogen metabolism processes[32,33]such as DNRA and ANRA[34–36]. In fact, four of the seven most abundant genera found in the cranberry fields are members of *Proteobacteria*. These include *Burkholderia* from the order *Burkholderiales* (class *Betaproteobacteria*), *Rhodoplanes* and *Bradyrhizobium* from the order *Rhizobiales* (class *Alphaproteobacteria*), and *Rhodanobacter* from the order *Xanthomonadales* (class *Gammaproteobacteria*). These genera are associated with nitrogen fixation, further supporting the role of *Proteobacteria* in enhancing nitrogen availability in cranberry soils[37]. The high abundance of these taxa in the L1 West side supports the hypothesis that subirrigated areas may harbor microbial communities more engaged in nitrogen transformation processes. Additionally, members of *Actinobacteria*, such as *Streptacidiphilus* and *Actinospica*, also present in our dataset, are known for their antagonistic activity against soilborne pathogens[29] and their potential role in nitrogen retention[35]. Rather than attributing these functions to entire phyla, our interpretation focuses on the ecological relevance of these dominant genera in the context of cranberry soil function.

Our study confirmed a distinct bacterial community response to variations in soil moisture, with DNRA-associated genes, such as *nirD*, showing a marked increase in side managed by subirrigation, which had lower moisture levels. This suggests that DNRA may be a key nitrogen conservation mechanism in drier soils, aligning with Pandey et al. (2020)[13]who observed that DNRA can proceed even under reduced moisture conditions, provided that organic matter is present and oxygen levels are low enough. In cranberry soils, slow decomposition of leaf litter with a high C: N ratio (~80:1) further supports DNRA[7,38]. As observed by Stackpoole et al. (2008)[7]only 43% of the leaf litter decomposed over a 2.5-year period, leaving a substantial amount of organic carbon in the soil. This organic matter serves as an electron donor for the DNRA process, promoting nitrogen retention as ammonium. One important factor is that lower soil moisture tends to reduce mineralization, the process by which organic nitrogen is converted into nitrate. Reduced mineralization means less nitrate is available for denitrification, potentially favoring DNRA, which can utilize even low nitrate levels to convert it into ammonium. Thus, in soils with controlled moisture, such as the subirrigated L1 W side, DNRA plays a crucial role in retaining nitrogen, leading to higher yields and improved nitrogen use efficiency.

These results reveal the importance of soil moisture conditions in regulating nitrogen transformation pathways in cranberry soils. The microbial functions potentially involved in this process were explored through functional inference based on 16 S rRNA gene sequences using the Tax4Fun2 tool. However, the use of Tax4Fun2 for functional prediction has certain limitations, including limited functional resolution and reliance on reference genome databases that may not accurately reflect the actual microbial diversity present in environmental samples. This approach is based on taxonomic similarity, which can introduce biases, particularly for microorganisms that are underrepresented in available databases[39,40]. Nevertheless, several studies have shown that Tax4Fun2 can provide useful exploratory insights into potential microbial functions, particularly in comparative analyses or for generating hypotheses in microbial ecology[40,41]. Thus, while acknowledging these limitations, we consider the approach to be complementary, offering a valuable preliminary functional overview within the context of our study, particularly for evaluating the potential contributions of microbial communities to nitrogen conservation via DNRA in cranberry soils under different water management practices. Future studies should aim to validate these predictions using metagenomic analyses, biogeographic surveys, and rhizotron-based experiments to better understand the relationship between DNRA activity and cranberry plant dynamics.

Why should we want to increase the DNRA function in cranberry soils? Increasing the dissimilatory nitrate reduction to ammonium (DNRA) function in cranberry soils presents several compelling advantages for enhancing cranberry production. One of the primary reasons is that cranberry plants preferentially absorb nitrogen in the form of ammonium, which is the final product of the DNRA process. By promoting DNRA activity, more nitrogen is retained in a stable, plant-available form (ammonium), rather than being lost through denitrification, which converts nitrate into nitrogen gases, including N_2O, a potent greenhouse gas[42]. This conservation of nitrogen not only supports plant growth but also reduces the reliance on synthetic nitrogen fertilizers, thereby lowering production costs and minimizing environmental impacts associated with fertilizer runoff and greenhouse gas emissions[43].

Research indicates that DNRA can significantly contribute to nitrogen retention in various ecosystems, including agricultural soils. DNRA and denitrification are two competing anaerobic microbial pathways that both use nitrate (NO_3^-) as the terminal electron acceptor, but they differ in their end products, ammonium (NH_4^+) for DNRA and N_2/N_2O for denitrification, as well as in their ecological implications (Kraft et al., 2014; Tiedje, 1988). For instance, studies have shown that DNRA processes are favored in environments with high carbon-to-nitrate (C: N) ratios, which enhance the efficiency of DNRA bacteria in utilizing nitrate[13,14,35,44,45]. This is particularly relevant for cranberry soils, which are often rich in organic material due to the slow decomposition of cranberry leaves, leading to a high C: N ratio[7]. While both DNRA and denitrification can be stimulated under high C: N conditions, the competition between these two pathways depends on additional environmental factors such as electron donor availability, redox conditions, and microbial community composition. In cranberry soils, organic matter accumulation may create conditions that selectively favor DNRA over denitrification, potentially due to factors like oxygen limitation and specific microbial populations adapted to DNRA.

Furthermore, the presence of specific microbial communities that facilitate DNRA is crucial for maintaining high rates of nitrogen retention[45]. By fostering these microbial populations, cranberry growers can enhance the soil's capacity to retain nitrogen, thus improving overall soil fertility and crop yield. Moreover, the reduction of N_2O emissions through enhanced DNRA activity is an essential environmental benefit whereas denitrification is a significant source of this GEG, which contributes to climate warming[46–48]. By shifting the nitrogen reduction pathway from denitrification to DNRA, the potential for GHG emissions can be significantly mitigated. This shift not only aligns with sustainable agricultural practices but also contributes to broader environmental services of reducing greenhouse gas emissions from agricultural systems[49].

Our study highlights the complex interactions between water management practices, specifically subirrigation, and soil bacterial communities in cranberry fields, with a particular focus on nitrogen cycling processes such as DNRA (dissimilatory nitrate reduction to ammonium). Subirrigation not only reduced water usage but also enhanced cranberry yields by promoting beneficial microbial groups, such as *Burkholderia* and *Arthrobacter*, that are associated with nitrogen conservation. These findings suggest that optimizing water management through subirrigation can foster a microbial environment conducive to higher cranberry productivity, offering both economic and environmental benefits, such as reduced reliance on synthetic nitrogen fertilizers and lower greenhouse gas emissions. However, despite the promising results, our study has several limitations. First, it is important to recognize that this was a case study conducted on only two cranberry fields, both of which had specific water table dynamics. The distinct water table depth (WTD) between the eastern and western sides of these fields led to natural moisture gradients, allowing us to investigate the effects of subirrigation. While this setup provided a unique opportunity to observe microbial and yield responses under different moisture conditions within the same agricultural system, it also limits the generalizability of our findings. The specific soil conditions and management practices in these fields may not represent the full diversity of cranberry cultivation systems, where soil types, climate, and water management practices can vary significantly. Moreover, our study relied heavily on predictive functional analyses of microbial communities, using 16 S rRNA sequencing to infer the presence of functional genes associated with DNRA and other nitrogen cycling pathways. While these predictions provide valuable insights, they do not directly measure the actual rates of nitrogen fluxes, such as DNRA activity or nitrogen loss through denitrification[50].

In conclusion, our study underscores the potential of subirrigation as a sustainable water management practice in cranberry production, particularly in promoting microbial communities that enhance nitrogen retention through DNRA. Future studies should incorporate more direct measurements of these processes, such as metagenomics, metatranscriptomics, or stable isotope probing, to confirm the functional roles of the microbial taxa identified in this study. Additionally, the long-term impacts of subirrigation on soil health, microbial community stability, and crop yield as well greenhouse gas emissions need further investigation. By deepening our understanding of the interactions between cranberry plants and soil microorganisms, we can move toward more resilient and sustainable cranberry production systems.

Materials and methods

Study area and soil sampling

The experimental cranberry fields (L1 and L3; Fig. 1A) are located at Saint-Louis-de-Blandford, Quebec, Canada (46°16'53.77"N; 71°57'46.61"W). These fields were specifically selected for the study due to their contrasting water table depths (WTD) on the east and west sides, which allowed us to examine the effects of subirrigation on soil moisture and microbial communities within a controlled agricultural environment. The fields are representative of typical cranberry production systems in Quebec, with drainage infrastructure designed to manage water levels and optimize cranberry yield. The presence of a natural soil moisture gradient between the E (conventional surface irrigation) and W (subirrigated) sides provided an ideal setting to study these effects.

A total of 36 soil samples was collected from each cranberry field, with 18 sampling points on each side of the respective field. To capture the variation in soil moisture conditions, 24 samples were taken directly above the drainage system, and 12 samples were collected between the drainage pipes (i.e., in the interdrain area) (Fig. 1B). Sampling was conducted in mid-September 2019, prior to harvest. This sampling strategy was designed to evaluate variations in soil moisture and microbial activity across different parts of the fields. The water table was consistently higher on the west side of each field, indicating that subirrigation was more effective in maintaining soil moisture in this region compared to the east where regional drainage was more important and subirrigation was insufficient to maintain the WTD. Therefore, the east side relied on conventional surface irrigation. This variation in WTD provided a valuable opportunity to study the impact of water management on microbial dynamics and crop yield.

Soil samples were collected at a depth of 0–25 cm using a soil core sampler. Each core was homogenized before being divided for subsequent analyses. Available nutrient levels were determined by the Mehlich III

method, conducted at the Research and Development Institute for the Agri-environment (RDIA) and included the analysis of various soil elements such as available phosphorus (P), magnesium (Mg), iron (Fe), potassium (K), calcium (Ca), and zinc (Zn). X-ray fluorescence spectrometry (X-MET8000) was also employed to determine the total concentrations of other elements such as silicon (Si), aluminum (Al), manganese (Mn), sulfur (S), and other trace elements. This method is particularly advantageous for determining total elemental concentrations, as it is non-destructive and requires minimal sample preparation, making it both efficient and accurate for environmental and agricultural applications. Finally, DNA was extracted for microbial community analysis.

DNA extraction and 16 S sequencing

For total DNA extraction and 16 S sequencing, we followed the protocol provided by the Earth Microbiome Project (EMP; www.earthmicrobiome.org). Total DNA was obtained from 0.25 g of soil using the PowerSoil DNA Isolation kit (MoBio, Carlsbad, USA) according to the manufacturer's instructions. The quality of extracted DNA was visualized by electrophoresis on a 0.8% agarose gel. The bacterial community sequences were obtained with the primers 515 F-806R, which target the V3-V4 side of the 16 S SSU rRNA[51]. The DNA library was generated using the Nextera XT Library Prep kit® with sequences of 300 base pairs, and the products were quantified using the Quant-iT PicoGreen dsDNA Assay Kit® (Thermo Fisher/Invitrogen cat. no. P11496; following the manufacturer's instructions) for equimolar pool tuning and sequenced using the MiSeq PE Cluster v3 cBot kit® (2 × 300 bp) on an Illumina MiSeq2500® platform (IRDA, Quebec, Canada).

Bioinformatics and statistical analysis

The bioinformatic analysis was performed in **R version 4.0.0** (R Core Team, 2020)[52] using the package **dada2** version 1.14.1, following the workflow proposed by Callahan et al. (2016)[53]. The first step involved verifying the sequences and removing primers using **cutadapt** version 2.10 (Martin, 2011)[54]. Next, filtering and trimming of bacterial reads were performed with truncLen = 240 and 170 bp for R1 and R2, respectively, with maxEE = 2 and maxN = 0 to control for sequence quality. Error learning was conducted using the **learnErrors** function in **dada2**, followed by dereplication using **derepFastq**. Sequence inference was performed with the **dada** function, and reads were merged with a minimum overlap of 12 bp. Chimeras were removed using the **removeBimeraDenovo** function, applying the "polled" method. To ensure accuracy, the method was evaluated using a mock community dataset, showing an exact match with the expected reference genomes for the 20 ASVs. Taxonomic assignment was conducted using the naive Bayesian classifier method with the **SILVA 132** database[55]. A total of 12,533 taxa (filtered to the Bacteria kingdom) were identified across the 72 soil samples, normalized without rarefaction following McMurdie and Holmes (2014)[56]ensuring comparability across samples.

Functional prediction of the microbial communities was performed using Tax4Fun2[40]. Predicted functional gene abundances were then correlated using both Pearson and Spearman correlation analyses to examine the relationships between genes involved in nitrogen cycling (e.g., DNRA, denitrification, nitrate assimilation) and soil attributes such as soil moisture, pH, and nutrient concentrations. Only interactions that presented significant correlations (p-value < 0.05) in both Pearson and Spearman analyses were retained for further interpretation. A network analysis was constructed to visualize the significant interactions between predicted genes and environmental factors, such as soil moisture and cranberry yield. The network was built using **Gephi 0.9.2**[57], and a fixed network layout was applied to display the relationships between genes and soil attributes. This visualization helped elucidate key gene interactions within the nitrogen cycle, highlighting those strongly associated with soil management practices like subirrigation.

For the statistical analysis, an **ANOVA** was performed to identify significant differences in soil moisture, soil chemistry, and cranberry yield between the east and west sides of the fields L1 and L3. The cranberry yield associated with each soil sample was interpolated with the "TPS" function in the "fields" package for R based on the cranberry yield at 80 points (40 points per field). The pairwise squared differences were evaluated with a traditional variogram ("vgram" R function) and cross-validated variogram ("crossCoVGram" R function). Although only two fields were studied, data were collected from numerous sampling points along both sides of the fields, allowing for a robust comparison. Average values for soil chemistry variables (e.g., Mg, Ca, K, Cu, Zn, Mn, Fe, P, pH), soil moisture, and cranberry yield were compared using post hoc Tukey tests (p-value < 0.05). **Principal component analysis (PCA)** was used to explore variation in soil chemistry, and linear regression models assessed correlations between cranberry yield and variables such as soil moisture, Mg, and Cu.

The **Shannon diversity index** was used to evaluate bacterial diversity. The relative bacterial abundance data had a non-normal distribution (Shapiro-Wilk test, p-value < 0.05), so the **Kruskal-Wallis rank-sum test** was used to compare average relative abundances. The bacterial community structure was evaluated with **Principal Coordinate Analysis (PCoA)**, distance-based redundancy analysis (**dbRDA**) and redundancy analysis (RDA) was performed to correlate bacterial community composition with environmental factors using the **Bray-Curtis dissimilarity index**. The significance of environmental predictors was assessed via PERMANOVA (adonis2, 999 permutations). Differential abundance analysis at the genus level was carried out using ANCOM-BC2, which models log-fold changes while accounting for data compositionality and sparsity. In this analysis, L1 East served as the reference group.

Data availability

The network analysis table, Pearson and Spearman correlation results, ASV table, soil chemistry data, and RF models are available at Figshare (https://figshare.com/s/3c989f2e028102f66eb4). Amplicon-sequencing files are available at NIH (PRJNA1203594; https://submit.ncbi.nlm.nih.gov/subs/sra/SUB14957935/)..

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Author contributions

A.K.A.N. contributed to the manuscript writing and finalization of data analyses. T.G. conducted bioinformatic and biostatistical analyses and was involved in all stages, including figure preparation. S.J.G. assisted with water table analyses and contributed to the data discussion. J.L. provided significant assistance with sampling and general data acquisition. P.C. contributed to the discussion, particularly regarding microbiological data. A.N.R. also assisted with water table analyses and contributed to data discussions. All authors reviewed the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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