Distribution Characteristics of Free-Living Nitrogen-Fixing Bacteria in Solonetz Soil Types

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ABSTRACT

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|  Solonetz soils, characterized by high sodium content and dense subsurface horizons, are globally distributed across arid and semi-arid regions, including parts of Central Asia, Eastern Europe, Australia, and South America. These soils pose significant challenges to agriculture due to poor structure, low permeability, and limited nutrient availability. Their high exchangeable sodium percentage negatively affects root growth and water infiltration, reducing crop productivity. Despite these constraints, understanding Solonetz soil dynamics is crucial for land reclamation and sustainable agriculture. This study investigates the solonetz soil type of Eastern Georgia, characterized by a pH of 6.42, moisture content of 12%, Salinity -0.8% , and a hydrolyzable nitrogen content of 5.2 mg per 100 g of soil. The study determined the population and genera composition of free-living nitrogen-fixing bacteria per gram of dry soil: Azotobacter (865,155; 22.27%), Beijerinckia (275,167; 7.08%), Azospirillum (2,678,150; 68.94%), and Derxia (65,750; 1.69%). Azospirillum was found to dominate, which correlates with low hydrolyzable nitrogen levels. The results suggest a probable link between the concentration of easily hydrolyzable nitrogen in soil and the distribution of nitrogen-fixing bacteria. |

*Keywords: Nitrogen, Soil, Solonetz, Qualitative, Quantitative.*

1. INTRODUCTION

 Soil nitrogen (N) dynamics are central to global agricultural productivity and environmental sustainability. Over the past several decades, human interventions—particularly the application of synthetic and mineral nitrogen fertilizers—have dramatically altered the global nitrogen cycle (Galloway et al., 2003;). While these inputs have contributed to enhanced crop yields and food security, they have also led to a cascade of ecological consequences, including nitrate leaching, eutrophication, and increased emissions of nitrous oxide (N2O), a potent greenhouse gas (Erisman et al., 2008; Sutton et al., 2011). Recognizing the unsustainability of current fertilization practices, the scientific community is increasingly turning to alternative, biologically based approaches for enhancing soil nitrogen status, with particular interest in the ecological roles of free-living nitrogen-fixing microorganisms (Vitousek et al., 2013).

Among biological nitrogen inputs, free-living diazotrophic bacteria represent a critical, yet underutilized, reservoir of nitrogen for terrestrial ecosystems. Unlike their symbiotic counterparts, which form intimate relationships with leguminous hosts, free-living diazotrophs can fix atmospheric nitrogen independently, colonizing a wide range of soil environments, including those considered marginal or degraded (Kennedy & Islam, 2001; Zehr & Turner, 2001). Notable genera in this group include Azotobacter, Azospirillum, and Beijerinckia, each exhibiting distinct physiological and ecological traits that enable them to survive and function across diverse edaphic conditions (Bashan & Holguin, 1997; Steenhoudt & Vanderleyden, 2000).

Azotobacter species, for example, are obligate aerobes known for their high respiratory rates, which help maintain intracellular anaerobic conditions suitable for nitrogenase activity despite the presence of oxygen. Their production of extracellular polysaccharides also provides resilience under drought and desiccation, enhancing survival in semi-arid and alkaline environments . Azospirillum spp., on the other hand, are microaerophilic and often associate with the rhizosphere of cereals and grasses, where they contribute not only to nitrogen fixation but also to plant growth through phytohormone production, particularly indole-3-acetic acid (IAA) (Dobbelaere et al., 2003; Okon & Labandera-Gonzalez, 1994). Beijerinckia spp. are typically found in acidic or oligotrophic soils, where their nitrogenase activity and acid tolerance make them valuable in soil restoration initiatives (Reinhold-Hurek & Hurek, 2000).

The significance of these genera is especially pronounced in saline-alkaline soils, such as solonetz, which present unique challenges to plant and microbial life due to their high pH, elevated sodium content, and poor physical structure (FAO, 2006; Szabolcs, 1989). These soils are common in arid and semi-arid regions and are often characterized by poor fertility and low organic matter content—factors that limit the success of conventional agriculture and reduce the efficacy of chemical fertilization. Consequently, biological nitrogen fixation via resilient microbial communities may represent one of the most viable pathways for restoring and sustainably managing solonetz soils.

Despite their inhospitable conditions, solonetz soils can support diazotrophic microbial communities, although with reduced diversity and activity compared to more fertile soils (Rousk et al., 2010). The persistence of Azotobacter in these environments has been attributed to its halotolerance and ability to form protective cysts, which shield cells from osmotic stress and desiccation (Bashan, 1998). Similarly, strains of Azospirillum isolated from saline soils often exhibit physiological adaptations such as compatible solute accumulation and modifications to membrane fluidity, enhancing their survival and metabolic function under high-salinity stress . Beijerinckia, while traditionally associated with acidic soils, has also been isolated from neutral to alkaline environments, suggesting a broader ecological amplitude than previously assumed .

The ecological performance of these bacteria in saline-alkaline soils depends on a range of factors, including soil pH, electrical conductivity (EC), organic matter availability, and plant-microbe interactions (Rietz & Haynes, 2003; Vessey, 2003). Soil salinity, in particular, poses a major constraint to microbial activity, often disrupting membrane potential and enzyme function (Ventosa & Nieto, 1995). However, the presence of organic substrates can mitigate these effects by providing energy sources that support microbial respiration and growth. In this context, the addition of organic amendments—such as compost, manure, or green manures—has been shown to stimulate diazotrophic activity, even in saline conditions (Peoples & Craswell, 1992; Kennedy et al., 2004).

Plant roots also play a central role in shaping microbial communities, especially in stress-prone soils. Through the release of root exudates, plants can selectively enrich for beneficial microorganisms, including nitrogen-fixing bacteria, in the rhizosphere and rhizoplane (Badri & Vivanco, 2009; Mendes et al., 2013). This interaction is particularly important in solonetz soils, where the presence of salt-tolerant vegetation can create microhabitats that buffer against salinity and promote microbial colonization. Studies have shown that certain halophytes and salt-tolerant grasses harbor diverse diazotrophic communities, with implications for revegetation and land reclamation efforts (Mapelli et al., 2013).

The distribution and abundance of diazotrophic bacteria in saline and alkaline soils also exhibit spatial and temporal variability. Vertical gradients in microbial community composition are often observed, with higher microbial densities and activity in surface layers where root exudates and organic residues are more concentrated (Fierer et al., 2003; Xu et al., 2014). Seasonal fluctuations in temperature and moisture further influence microbial dynamics, often reducing activity during hot, dry periods and promoting growth during cooler, wetter seasons (Bell et al., 2009). These factors must be considered when evaluating the potential of nitrogen-fixing bacteria to contribute to soil fertility in solonetz environments.

Although the agronomic benefits of inoculating crops with Azotobacter and Azospirillum are well documented in conventional systems, their use in saline-alkaline soils remains limited and context-dependent. Field trials have demonstrated variable outcomes, influenced by local soil conditions, microbial compatibility, and crop species (Boddey et al., 2003; Bhardwaj et al., 2014). Nonetheless, integrated approaches that combine microbial inoculation with organic amendments and salt-tolerant crop varieties hold promise for enhancing nitrogen inputs and improving soil health in degraded lands.

In this context, the exploration of free-living diazotrophs in solonetz soils is not only of theoretical interest but also of practical relevance. Understanding the ecological strategies that enable these microbes to persist and function under extreme conditions can inform the development of bio-based soil management practices tailored to saline-alkaline environments. Such knowledge is essential for advancing sustainable agriculture, particularly in regions where chemical inputs are economically or environmentally unfeasible. The current study aims to address this need by investigating the abundance, diversity, and ecological roles of nitrogen-fixing bacteria—especially Azotobacter, Azospirillum, and Beijerinckia—in nitrogen-deficient solonetz soils. Through this work, we seek to contribute to the broader effort of reducing reliance on synthetic fertilizers while promoting biologically mediated soil fertility restoration.

2. material and methods

Soil samples from the solonetz soil type of Eastern Georgia were collected using the envelope method. Soil moisture, salinity, and pH were measured using a pH meter (Ohaus Starter 2100). The enumeration of bacteria was carried out using the serial dilution method, with results calculated per gram of dry soil.

\*\*Determination of Hydrolyzable Nitrogen\*\*
Easily hydrolyzable nitrogen was measured using the Tyurin and Kononova method.

\*\*Bacterial Identification\*\*
Microbiological methods commonly accepted in the field were employed for bacterial identification.

\*\*Culture Media\*\*
- \*\*Ashby’s Medium\*\*: Ingredients (g/L): Mannitol 20, K₂HPO₄ 0.2, MgSO₄ 0.2, NaCl 0.2, K₂SO₄ 0.1, CaCO₃ 5.0, Agar 15.0; Final pH 7.4 ± 0.2 .

- \*\*Beijerinckia Medium\*\*: Ingredients (g/L): KH₂PO₄ 0.8, Sucrose 20.0, K₂HPO₄ 0.2, MgSO₄ 0.5, FeCl₃ 0.1, Na₂MoO₄ 0.005, Agar 15.0; Final pH 6.5 ± 0.2 .

-\*\*Nitrogen-Free Glucose-Enriched Agar\*\*: Ingredients (g/L): KH₂PO₄ 1.0, CaCl₂ 1.0, MgSO₄·7H₂O 0.25, NaCl 0.5, FeSO₄·7H₂O 0.01, MnSO₄·H₂O 0.01, Na₂MoO₄ 0.01, Glucose 7.0,Agar20.0 .

- \*\*Burk’s Medium\*\*: Ingredients (g/L): MgSO₄ 0.2, K₂HPO₄ 0.8, KH₂PO₄ 0.2, CaSO₄ 0.13, FeCl₃ 0.00145, Na₂MoO₄ 0.000253, Sucrose 20.0 .

- \*\*Azospirillum Medium\*\*: Ingredients (g/L): C₄H₆O₅ 5.0, K₂HPO₄ 0.5, FeSO₄ 0.5, MnSO₄·H₂O 0.01, MgSO₄ 0.2, NaCl 0.1, Bromothymol Blue 0.002, Na₂MoO₄ 0.002, CaCl₂ 0.02, Agar 1.75, KOH 4.0; Final pH 6.8 ± 0.2 .

To test the ability to produce ammonia, we inoculated the microbes in sterile water with a carbon source, and after 48 hours of cultivation at 28 degrees, we checked the color yellowness with Nessler's reagent. Then, biochemical studies of nitrogen-fixing bacteria were performed to identify them to the genus level.Test for urease activity

  

**FIGURE 1. Test for urease activity**

Test for urease activity

plated the isolated cultures on the urea agar test medium available in the laboratory. After 24 hours, observed the color change.

Catalase test

 pour 3% hydrogen peroxide into a sterile cup and insert a 24-48 hour bacterial culture with a loop. If bubbles appear within three minutes, the catalase assay is positive.

Oxidase test

 used the oxidase plates available in the laboratory, on which we inserted 24 hour (37 degrees) colonies with a loop. If a purple color develops within 10 seconds, this indicates a positive test.

 used the motility tests available in the laboratory, the culture was inserted into the center of the semi-solid agar with a needle. If growth was only in those places where we passed the needle, this indicated the immobility of the microbe. A repeat test was performed after 7 days.

The isolated nitrogen-fixing bacteria were tested on 6 sugars. Phenol red was used as a color indicator.

Determination of soil salinity by the porcelain cup extraction method

For the initial assessment of the degree of soil salinity, used the porcelain cup extraction method, which is based on the slow steam drying of the aqueous extract obtained from the soil and the visual assessment of the resulting saline precipitate.

Methodology:

For the experiment, selected an air-dried soil sample passed through a 1 mm sieve. weighed 50 grams of soil and placed it in a chemically clean glass container. Then we added 50 ml of distilled water (depending on the situation, the mixing ratio was 1:1). stirred the mixture with a glass rod and left it for 1 hour so that the soluble salts could completely pass into the aqueous phase.

After the time elapsed, filtered the extract through filter paper and obtained a clear liquid. placed 5 ml of this extract in a clean porcelain beaker and dried it with slow steam in a warm, dust-free environment (~50°C) to prevent leaching of salts or chemical degradation.

After drying, a clearly visible white crystalline precipitate appeared on the inner surface of the beaker. The density and uniform distribution of salts indicated a moderately saline soil. The result obtained corresponds to a relatively high concentration of soluble salts, which may indicate the presence of sodium and calcium chlorides and sulfates. After evaporating the extracts from all soils and all depths, recalculated their percentages according to the method.

Determination of Soil Moisture Content Using the Drying Oven Method

To determine the moisture content of the soil samples, used the standard drying oven method. For each sample, took a clean and dry crucible (weighing dish), recorded its initial weight (M₀), and then added approximately 10 grams of fresh (wet) soil. We recorded the combined weight of the crucible and moist soil (M₁).

Next, we placed the open crucibles in a drying oven set at 105 °C and left them for approximately 12 hours, or until constant weight was achieved. After drying, transferred the crucibles into a desiccator to cool and prevent moisture absorption from the air.

Once cooled, we weighed the crucibles again to determine the mass of the dry soil (M₂). Using these measurements, calculated the moisture content of each soil sample using the following formula:

Using this method, successfully determined the moisture content for all the soil samples under investigation.

Readily hydrolyzable nitrogen is determined by the Turen-Kononova method.

3. results and discussion

The study analyzed the soil’s pH (6.42), moisture content (12%) Salinity -0.8% , and hydrolyzable nitrogen (5.2 mg/100 g of dry soil). The total count of nitrogen-fixing bacteria was 3,884,222 per gram of dry soil. The genera composition was as follows: Azospirillum (68.94%), Azotobacter (22.27%), Beijerinckia (7.08%), and Derxia (1.69%).

The data indicates that Eastern Georgia’s solonetz soil type is characterized by lower pH and hydrolyzable nitrogen compared to other soil types (e.g., gray-brown, meadow-brown, and alluvio-carbonate soils, with pH values of 6.9, 6.5, and 6.64, respectively).

This analysis reveals that low nitrogen levels positively influence the proliferation of nitrogen-fixing bacteria, particularly favoring Azospirillum.

**Table 1 Distribution of Nitrogen-Fixing Bacteria in solonetz Soil.**

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| --- | --- | --- |
| **Genera** | **Quantity (CFU/g)** | **Percentage (%)** |
| Azotobacter | 865,155 | 22.27% |
| Beijerinckia | 275,167 | 7.08% |
| Azospirillum | 2,678,150 | 68.94% |
| Derxia | 65,750 | 1.69% |

**Table 2 Soil physicochemical parameters**

|  |  |
| --- | --- |
| pH |  6.42 |
| Hydrolyzable nitrogen | 5.2 |
| MoistHydrolyzing nitrogen  | 12% 5.2mg |

In soils with similar pH (6.43) and moisture levels (13%), such as mountain-meadow soils, higher hydrolyzable nitrogen (14-30 mg/100 g of soil) and significantly lower nitrogen-fixing bacteria counts (250 CFU) are observed, predominantly Azotobacter.

This analysis reveals that low nitrogen levels positively influence the proliferation of nitrogen-fixing bacteria, particularly favoring Azospirillum.

 The results of the study show that this type of soil has favorable physicochemical parameters that are favorable for nitrogen fixers of the genus Azospirillum. Presumably, due to the low amount of hydrolyzable nitrogen in this type of soil, the nitrogen fixation process should proceed strongly, despite the acidic pH. Based on all of this, It seems that the small amount of plant material present in this type of soil produces a sufficient amount of exudate for Azospirillum. The high activity of Azospirillum (68.94%) in the untreated, fertilized Solonetz soil can be explained by several interconnected factors. This bacterium is a rhizosphere-oriented nitrogen fixer that colonizes the root surface or even penetrates inside the roots and responds well to plant exudates. In untreated soil, the natural plant cover continuously supplies such exudates, which promotes the proliferation of Azospirillum. At the same time, Solonetz soils are characterized by sodium ion stress, limited aeration, and poor water infiltration, which restrict the activity of other free-living nitrogen-fixing bacteria such as Azotobacter, Beijerinckia, and especially Derxia. Under these stressful conditions, Azospirillum is comparatively tolerant and occupies the ecological niche where competition is low. The low level of hydrolyzable nitrogen in the soil (5.2 mg/100 g) indicates a scarcity of organic matter, which further favors bacteria capable of using plant exudates as an energy source, while free-living forms are more dependent on the presence of organic compounds in the soil. As a result, the natural nitrogen fixation potential of this soil is predominantly provided by the rhizospheric, stress-tolerant Azospirillum, whereas other genera remain in a minor position due to the chemical and physical stress characteristic of Solonetz soils.

the use of Azospirillum as a biofertilizer would likely be a good option for increasing the nitrogen pool in this type of soil. It seems that the conditions in this soil are favorable for Azospirillum, and it is possible that a significant portion of the nitrogen in the soil is produced by it.

4. Conclusion

The Solonetz soil type in Eastern Georgia has a pH of 6.42, a moisture content of 12%, Salinity -0.8% and hydrolyzable nitrogen content of 5.2 mg per 100 g of soil.

The total population of nitrogen-fixing bacteria in this soil is 3,884,222 per gram of dry soil, with the genera distributed as follows: Azotobacter (22.27%), Beijerinckia (7.08%), Azospirillum (68.94%), and Derxia (1.69%).

The concentration of easily hydrolyzable nitrogen appears to determine both the quantitative distribution of nitrogen-fixing bacteria and the composition of individual genera

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.

2. Chat gpt was used to find the latest literature, in case of an error, the fault of the Ai system is present.

References

Badri, D. V., & Vivanco, J. M. (2009). Regulation and function of root exudates. *Plant, Cell & Environment*, 32(6), 666–681.

Bashan, Y. (1998). Inoculants of plant growth-promoting bacteria for use in agriculture. *Biotechnology Advances*, 16(4), 729–770.

Bashan, Y., & Holguin, G. (1997). Azospirillum–plant relationships: Environmental and physiological advances (1990–1996). *Canadian Journal of Microbiology*, 43(2), 103–121.

Bell, C., McIntyre, N. E., Cox, S., Tissue, D. T., & Zak, J. C. (2009). Soil microbial responses to temporal variations of moisture and temperature in a Chihuahuan Desert grassland. *Microbial Ecology*, 58(4), 827–842.

Bhardwaj, D., Ansari, M. W., Sahoo, R. K., & Tuteja, N. (2014). Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. *Microbial Cell Factories*, 13(1), 66.

Boddey, R. M., Urquiaga, S., Alves, B. J. R., & Reis, V. (2003). Endophytic nitrogen fixation in sugarcane: Present knowledge and future applications. *Plant and Soil*, 252(1), 139–149.

Dobbelaere, S., Vanderleyden, J., & Okon, Y. (2003). Plant growth-promoting effects of diazotrophs in the rhizosphere. *Critical Reviews in Plant Sciences*, 22(2), 107–149.

Erisman, J. W., Sutton, M. A., Galloway, J., Klimont, Z., & Winiwarter, W. (2008). How a century of ammonia synthesis changed the world. *Nature Geoscience*, 1(10), 636–639.

FAO. (2006). *World reference base for soil resources 2006: A framework for international classification, correlation and communication*. Food and Agriculture Organization of the United Nations.

Fierer, N., Schimel, J. P., & Holden, P. A. (2003). Influence of drying–rewetting frequency on soil bacterial community structure. *Microbial Ecology*, 45(1), 63–71.

Galloway, J. N., Aber, J. D., Erisman, J. W., Seitzinger, S. P., Howarth, R. W., Cowling, E. B., & Cosby, B. J. (2003). The nitrogen cascade. *BioScience*, 53(4), 341–356.

Kennedy, I. R., & Islam, N. (2001). The current and potential contribution of asymbiotic nitrogen fixation to nitrogen requirements on farms: A review. *Australian Journal of Experimental Agriculture*, 41(3), 447–457.

Kennedy, I. R., Choudhury, A. T. M. A., & Kecskés, M. L. (2004). Non-symbiotic bacterial diazotrophs in crop-farming systems: Can their potential for plant growth promotion be better exploited? *Soil Biology and Biochemistry*, 36(8), 1229–1234.

Mapelli, F., Marasco, R., Rolli, E., Barbato, M., Cherif, A., Guesmi, A., ... & Daffonchio, D. (2013). Potential for plant growth promotion of rhizobacteria associated with Salicornia growing in Tunisian hypersaline soils. *BioMed Research International*, 2013, 248078.

Mendes, R., Garbeva, P., & Raaijmakers, J. M. (2013). The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiology Reviews*, 37(5), 634–663.

Peoples, M. B., & Craswell, E. T. (1992). Biological nitrogen fixation: Investments, expectations and actual contributions to agriculture. *Plant and Soil*, 141(1-2), 13–39.

Reinhold-Hurek, B., & Hurek, T. (2000). Reassessment of the taxonomic structure of the diazotrophic genus Azoarcus and its relation to the beta-Proteobacteria. *International Journal of Systematic and Evolutionary Microbiology*, 50(3), 1103–1113.

Rietz, D. N., & Haynes, R. J. (2003). Effects of irrigation-induced salinity and sodicity on soil microbial activity. *Soil Biology and Biochemistry*, 35(6), 845–854.

Rousk, J., Brookes, P. C., & Bååth, E. (2010). Investigating the mechanisms for the opposing pH relationships of fungal and bacterial growth in soil. *Soil Biology and Biochemistry*, 42(6), 926–934.

Steenhoudt, O., & Vanderleyden, J. (2000). Azospirillum, a free-living nitrogen-fixing bacterium closely associated with grasses: Genetic, biochemical and ecological aspects. *FEMS Microbiology Reviews*, 24(4), 487–506.

Sutton, M. A., Howard, C. M., Erisman, J. W., Billen, G., Bleeker, A., Grennfelt, P., ... & Grinsven, H. V. (2011). *The European nitrogen assessment: Sources, effects and policy perspectives*. Cambridge University Press.

Szabolcs, I. (1989). *Salt-affected soils*. CRC Press.

Ventosa, A., & Nieto, J. J. (1995). Biotechnological applications and potentialities of halophilic microorganisms. *World Journal of Microbiology and Biotechnology*, 11(1), 85–94.

Vessey, J. K. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil*, 255(2), 571–586.

Vitousek, P. M., Menge, D. N. L., Reed, S. C., & Cleveland, C. C. (2013). Biological nitrogen fixation: Rates, patterns and ecological controls in terrestrial ecosystems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368(1621), 20130119.

Xu, X., Thornton, P. E., & Post, W. M. (2014). A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. *Global Ecology and Biogeography*, 23(7), 733–749.( YEAR NOT MATCH)

Zehr, J. P., & Turner, P. J. (2001). Nitrogen fixation: Nitrogenase genes and gene expression. In *Methods in Microbiology* (Vol. 30, pp. 271–286). Academic Press.ID).