Transcriptional Responses and Microbiome Shifts in Pea Roots Under High pH Stress

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Abstract

High soil pH negatively impacts growth and productivity, yet the transcriptomic profile and microbial dynamics underlying the stress response in garden pea (*Pisum sativum* ssp. hortense) remain elusive. In this study, high pH in soil caused stunted growth and biomass reduction, accompanied by impaired photosynthesis and chlorophyll synthesis. We observed shifts in key nitrogen-fixing bacteria, including *Rhizobium indicum*, *R. leguminosarum*, and *R. redzepovicii*, in nodules under high pH, highlighting the importance of micronutrient balance in legume-microbe symbiosis. This was accompanied by the downregulation of *NifA* and *NifD* genes, though *NifH* upregulation suggests a compensatory response to maintain nitrogenase activity. RNA-seq analysis unveiled extensive transcriptional reprogramming, with upregulation of genes associated with oxidative stress response (oxidoreductase activity, *Psat1g015240*; glutathione transferase activity, *Psat1g010280*), metal ion transport (*Psat1g011640*), and downregulation of ammonia-lyase activity (Psat1g046920) and ion binding (Psat1g040440, Psat5g094200) genes suggests broader changes in nutrient balance and homeostasis under high pH conditions. KEGG pathway analysis revealed metabolic constraints and potential disruptions in stress signaling pathways, such as the MAPK signaling pathway. Amplicon sequencing analysis reveals significant shifts in the diversity and abundance of root-associated bacterial communities in response to high pH. We observed the enrichment of *Variovorax*, *Shinella*, and *Methylophilus* in the roots, suggesting their potential recruitment by host determinants in response to high pH. This increase in siderophore production suggests a microbial strategy to cope with micronutrient imbalance in high pH. Additionally, stable genera such as *Pseudomonas*, *Novosphinogobium*, *Mycobacterium*, and *Herbaspirillum* indicate resilience to soil alkalinity, potentially serving as key components of the core microbiome in adapting to high pH envFements. Our findings will lay the groundwork for exploring alkali-resistant genes and enriched microbiomes, potentially aiding in the breeding program and development of microbial consortia to help pea plants cope with high-pH soils.

Keywords: Soil pH, legume crop, nitrogen fixation, enriched microbiome, soil alkalinity, transcriptome.

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INTRODUCTION

High pH, or alkaline conditions, can significantly impact plant growth and micronutrient availability (Xia et al. 2024). The elevated levels of Na^+, HCO^{-3}, and CO_2^{-3} in soils contribute to an increase in soil pH (Li et al. 2018). In alkaline soils, the solubility of many essential micronutrients, such as iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) decreases. These micronutrients act as necessary components for various enzymes and proteins, which are pivotal for numerous physiological functions such as photosynthesis, protein synthesis, nitrogen metabolism, and respiration (Kaya et al. 2019). The amount of micronutrients, especially Fe that plants can access in the soil is insufficient for their growth requirements, particularly in alkaline soil conditions, leading to High pH (Zhu et al. 2019). High pH conditions primarily manifest in plants through symptoms such as chlorosis, stunted growth, necrosis, distorted growth patterns, root dysfunction, increased susceptibility to diseases, and altered coloration, all of which are linked to impaired nutrient uptake, particularly of micronutrients like Fe, zinc, and manganese (Turner et al. 2020; Samborska-Skutnik et al. 2020). Thus, alkalinity-induced micronutrient deficiency can greatly hamper the structure and operation of photosynthetic machinery in plants (Singh et al. 2021). Thus, enhancing the tolerance of rice to alkaline stress is crucial for achieving consistently high and stable yields.

Plants cope with soil alkalinity through various strategies that enhance their resilience and nutrient uptake. They often release organic acids from their roots to lower the pH around the root zone, improving the availability of essential nutrients (Barrow and Hartemink, 2023; Ma et al. 2022). Physiologically, plants employ specialized ion transport mechanisms to uptake beneficial cations, and they may regulate their internal pH using proton pumps (Ku et al. 2022; Kabir et al. 2012). Morphologically, some species develop extensive root systems to access deeper soil layers and establish mycorrhizal associations, enhancing nutrient absorption (Zhang et al. 2020; Chen et al. 2016). Particularly in high pH-induced Fe deficiency, dicot plants are characterized by an increase in the activity of a plasma membrane-bound reductase, which leads to higher rates of Fe(III) reduction and the subsequent breakdown of Fe(III)-chelates at the plasma membrane (Kabir et al. 2012; Marschner et al. 1986). Plant roots may release reducing and/or chelating substances (e.g., phenolics) into the rhizosphere, further mobilizing sparingly soluble micronutrients under soil alkalinity (Wu et al. 2012). Furthermore, plants utilize a variety of genes to cope with soil alkalinity, facilitating their adaptation to high pH conditions. Ion transporter genes, including H^+-ATPases and Na^+/H^+ antiporters, help maintain ion homeostasis by regulating the uptake of essential cations while preventing toxic sodium accumulation (Assaha et al. 2017). Additionally, ferric reductase genes like *FRO2* are upregulated to reduce Fe(III) to Fe(II), aiding Fe uptake under alkaline conditions (Kabir et al. 2012).

In legume plants, the nodulation process initiates through a symbiotic interaction with nitrogen-fixing bacteria, leading to the formation of root nodules. These structures are crucial as they house the nitrogenase enzyme responsible for converting atmospheric nitrogen into a usable form for plants (Wang et al. 2018). High pH-induced High pH can impair the formation and function of these nodules, leading to reduced nitrogen fixation and overall plant health (Sankari et al.

2022; Oláh et al. 2001). The availability of Fe plays a crucial role in the biosynthesis of several proteins essential for nodulation (Brear et al. 2013; Abadía, 2000). Nodulation bacteria, primarily rhizobia, play a crucial role in the nitrogen fixation process in legume crops (Shumilina et al. 2023; Dusha, 2002). Studies have revealed that insufficient Fe levels due to soil alkalinity can hinder both the growth of legume crops and their capacity for symbiotic nitrogen fixation (Slatni et al. 2011). Furthermore, high pH can lead to increased competition from symbiotic bacteria and changes in the microbial community structure in the rhizosphere (Deng et al. 2024). Although limited research has been conducted on the dynamics of bacterial communities in plants exposed to high pH, carbonate ions have been shown to significantly affect rhizosphere fungal communities (Luo et al. 2023).

Garden pea (*Pisum sativum* ssp. hortense) is an important source of micronutrients for both humans and animals (Dahl et al. 2012). However, high soil pH in peas results in significant yield loss due to impaired chlorophyll production, leading to chlorosis and stunted growth (Meisrimler et al. 2016). However, the impact of high pH on plant-microbe interactions and the underlying transcriptional response in the roots remains unclear in garden pea. Therefore, this study aims to investigate the physiological responses, transcriptional alterations, and bacterial diversity and abundance in the nodules and roots of pea plants subjected to high pH. This research could lead to the identification of marker genes for breeding pea cultivars and formulations of biofertilizers with enhanced tolerance to high pH in soil.

MATERIALS AND METHODS

Plant cultivation and growth conditions

The seeds of garden peas (var. Sugar Snap) underwent surface sterilization using a 4% sodium hypochlorite solution for 5 min, followed by three washes with sterile water. Subsequently, they were placed in a tray and incubated at 25°C under dark for 2 d to induce germination. Once uniform and healthy seedlings emerged in 3d, they were transplanted into soil pots (500 g soil), consisting of a mixture of natural field soil and commercial potting mix in a 1:1 ratio, for two distinct treatments: control (soil without lime, pH ~6.5) and high pH (soil amended with 4.5 g $NaHCO_3$ and 3.0 g $CaCO_3$, pH ~7.8). Throughout the cultivation period, 15 mM $NaHCO_3$ was added weekly to the high-pH soil to maintain its pH to 8.0 (Kabir et al. 2012). The physicochemical characteristics and nutritional status of the soil are detailed in Supplementary Table S1. The plants were arranged in a randomized complete block design with 9 replications per treatment and placed in a growth chamber with a photoperiod of 10 h of light and 14 h of darkness (250 μmol m^{-2} s^{-1}) at approximately 25°C for 5 weeks. The plants were irrigated with deionized water every other day.

Measurement of morphological features and chlorophyll fluorescence kinetics

The shoot height and longest root of each plant were assessed using a measuring tape. Subsequently, the harvested shoot and root were subjected to a drying process for 3 d at 70°C in an electric oven before their dry weights were recorded. Additionally, the roots underwent a gentle washing procedure to eliminate soil particles. Nodules were then meticulously harvested from the root system, counted using a magnifying lens, and stored in an -80°C freezer for subsequent analysis.

Furthermore, chlorophyll scoring and chlorophyll fluorescence kinetics (OJIP) measurements, including Fv/Fm (maximal photochemical efficiency of PSII), Pi_ABS (photosynthesis performance index), and Mo (approximated initial slope in ms-1 of the fluorescent transient), were conducted on the uppermost fully developed leaves at three different locations using a handheld chlorophyll meter (AMTAST, United States) and FluorPen FP 110 (Photon Systems Instruments, Czech Republic), respectively. For OJIP analysis, the leaves were acclimated to darkness for 1 hour before data collection.

Nutrient analysis in root and shoot

Nutrient analysis was carried out on both the roots and young leaves. To begin, the roots were detached, washed with running tap water, and then submerged in 0.1 mM $CaSO_4$ solution for 10 min. Subsequently, they were rinsed with deionized water. The young leaves were washed separately with deionized water. Following this, the samples were individually placed in envelopes and subjected to drying in an electric oven at 75°C for 3 d. Elemental concentrations were measured using inductively coupled plasma mass spectrometry (ICP-MS) at the Agricultural & Environmental Services Laboratories at the University of Georgia. The concentrations were determined by comparing the intensity of the ion peaks in the spectrum to calibration standards.

RNA-sequencing and bioinformatics analysis

RNA-seq analysis was conducted on root samples. Before RNA extraction, the roots were meticulously cleaned by washing twice in sterile phosphate-buffered saline with 10 sec of vortexing, followed by two rinses with sterile water to remove surface contaminants. The cleaned samples were then ground to a powder using a pre-cooled pestle and mortar with liquid nitrogen. The powdered material was used for RNA extraction, which was carried out using the SV Total RNA Isolation System according to the manufacturer's protocol (Promega, USA). For each sample, 1 μg of RNA with an RIN value greater than 8 was utilized as input material for RNA-seq library preparations. The sequencing libraries were generated using the KAPA HiFi HotStart Library Amplification Kit (Kapa Biosystems, USA). The libraries underwent sequencing on an Illumina NovaSeq 6000 instrument using 0.2 Shared S4 150 bp paired-end sequencing at RTSF Genomics Core at Michigan State University. Overall, 92.5% of reads passed filters with a quality score of ≥ Q30.

Bioinformatics analysis of raw FastQ files was performed using Partek Flow genomic analysis software (Partek, St. Louis, MO, USA). Adaptor sequences and low-quality reads were removed from the raw data using Trimmomatic (Bolger et al. 2014) to obtain high-quality clean reads. Reference genome for garden pea was downloaded and the clean reads were aligned to these genomes using HISAT2 (v2.0.5) (Kim et al. 2015). Gene read counts were determined using HTSeq (Anders et al. 2015). Differentially expressed genes (DEGs) were identified with the DESeq2 R package (v1.16.1), calculating FPKM (fragments per kilobase of exon per million mapped fragments). DEGs were defined as those with an adjusted P-value < 0.05 and a fold change ≥ 2, using the false discovery rate (FDR) method. Venn diagrams and heatmaps were created using VennDetail (Hinder et al. 2017) and pheatmap (Hu et al. 2021) in R, respectively. Gene ontology and enrichment analyses of garden pea DEGs were conducted with ShinyGO 0.76.3 (Ge et al. 2020), utilizing gene ontology data from EnsemblPlants resources (Yates et al. 2022).

Nanopore-based 16S sequencing in root nodules

The isolated nodules underwent a thorough washing with running tap water, followed by surface sterilization in a 3% sodium hypochlorite solution for 3 min, and then rinsed three times with sterilized distilled water. Total DNA extraction was performed using the CTAB method as previously described (Clarke, 2009). For PCR reactions, 5 ng of DNA from each sample was used with 16S primers 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (CGGTTACCTTGTTACGACTT) for amplification of the near full-length bacterial 16S rRNA gene. PCR was carried out with the following thermal cycling conditions: initial denaturation at 95°C for 2 min, followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 60°C for 30 sec, and extension at 72°C for 2 min. This was succeeded by a final extension at 72°C for 10 min and a hold at 4°C. Amplicons from each sample were ligated to pooled barcoded reads using the 16S Barcoding Kit 24 V14 (SQK-16S114.24, Oxford Nanopore Technologies, Oxford, UK) for library construction. Sequencing was performed on a MinION portable sequencer (Oxford Nanopore Technologies) using a flow cell (R10.4.1).

The MinION™ sequencing data (FASTQ files) were processed using the EPI2ME software from Oxford Nanopore Technologies to generate pass reads in FASTQ format with an average quality score exceeding 9. The EPI2ME Fastq Barcoding workflow (Oxford Nanopore Technologies) was employed for trimming adapter and barcode sequences. Principal coordinate analysis, alpha diversity, and relative abundance of the bacterial community were computed using the R packages phyloseq and vegan. Non-parametric Kruskal–Wallis tests were applied to detect differences in taxa abundance at a significance level of 5% (McMurdie and Holmes, 2013).

Real-time qPCR analysis of nitrogen-fixing genes in root nodules:

Before RNA extraction from pea nodules, we thoroughly removed the surface contaminants by washing them with PBS buffer. RNA extraction and cDNA synthesis were performed followed by SV Total RNA Isolation System (Promega Corporation, USA) and GoScript™ Reverse Transcriptase (Promega Corporation, USA) kits, respectively. The expressions of *NifA* (nitrogenase Fe protein regulatory protein), *NifD* (nitrogenase FeMo protein alpha subunit), and *NifH* (Nitrogenase FeMo protein beta subunit) associated with the nitrogen fixation process in diazotrophic bacteria in nodules were then studied using gene-specific primers (Supplementary Table S1) with the CFX96 Real-Time system (Bio-Rad, USA). The qPCR reaction was conducted under the following conditions: initial denaturation at 95°C for 3 min, followed by 40 cycles consisting of denaturation at 95°C for 5 sec, annealing at 56°C for 30 sec, and extension at 60°C for 5 min. Expression levels of target genes were analyzed using the $dd^{-\Delta Ct}$ method (Livak and Schmittgen, 2001), with normalization to *PsGAPDH*.

Amplicon sequencing of 16S bacterial community

Amplicon sequencing targeting the 16S rRNA gene was conducted to analyze microbial communities in the root samples. The root cleaning protocol involved vortexing the samples in sterile phosphate-buffered saline (PBS) for 10 sec, followed by two rinses with sterile water to eliminate surface contaminants. Approximately 0.2 g of cleaned root tissue was utilized for DNA extraction, adhering to the manufacturer's instructions provided with the Wizard® Genomic DNA Purification Kit (Promega Corporation, United States). This extraction process included

treatments with RNase and Proteinase K to remove potential RNA and protein contaminants, respectively.

The amplicon library for the 16S rRNA gene was prepared using the primer pair 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC). Sequencing was conducted on the Illumina MiSeq platform using a paired-end read length of 250 base pairs. Raw sequencing reads were processed and trimmed using the cutadapt software (Martin, 2011), followed by analysis with the DADA2 pipeline (Callahan et al. 2016). Sequences identified as originating from mitochondria and chloroplasts were excluded from the dataset. Taxonomic assignments for amplicon sequence variants (ASVs) were conducted using the UNITE fungal reference database (Nilsson et al. 2019) for internal transcribed spacer (ITS) regions and version 138 of the Silva Project (Quast et al. 2013) for 16S rRNA sequences. Principal Coordinate Analysis (PCoA) was performed by calculating Bray-Curtis distance matrices based on Hellinger-transformed counts, utilizing the phyloseq and vegan packages in R. Non-parametric Kruskal-Wallis tests were employed to assess differences in taxa abundance. ASVs were classified into various taxonomic levels, and comprehensive data analyses—including principal component analysis, alpha diversity, beta diversity, and relative abundance assessment—were conducted at a significance threshold of $P < 0.05$ using the phyloseq package in R (McMurdie and Holmes, 2013).

Determinations of siderophore levels in the rhizosphere

We quantified the siderophore content in the rhizosphere soil using a chrome azurol S (CAS) assay, following the procedure outlined by Himpsl and Mobley (2019). In summary, rhizosphere soil was homogenized with 80% methanol and subsequently centrifuged for 15 min at 10,000 rpm. The resulting supernatant (500 µl) was mixed with 500 µl of CAS solution and allowed to incubate at room temperature for 5 min. The absorbance of each reaction was then measured at 630 nm against a reference containing 1 ml of CAS reagent alone. The siderophore level per gram of soil sample was calculated using the formula: % siderophore unit = [(Ar - As) / Ar] x 100, where Ar represents the absorbance at 630 nm of the reference (CAS reagent only), and As represents the absorbance at 630 nm of the sample (CAS reagent + soil sample).

Data analysis

All data were collected from three independent biological replicates for each treatment. The statistical significance of each variable was assessed through Student's *t-test*. Graphical presentations were prepared using ggplot2 in R program.

RESULTS

Morphological and nutrient analysis

The cultivation of garden peas exposed to high pH resulted in impaired aboveground growth (Fig. 1A). The garden pea plants showed a significant decline in root length, root dry weight, shoot height and shoot dry weight due to high pH relative to untreated controls (Fig. 1B-E). ICP-MS analysis showed a significant decrease in Fe, Zn, Mn, and S in both root and shoot of pea plants exposed to high pH in contrast to controls (Table 1). However, Mg and Ca levels exhibited no significant alterations in response to high pH in comparison to controls (Table 1).

Measurement of chlorophyll score and photosynthetic performance

Chlorophyll scores and chlorophyll fluorescence parameters were evaluated to elucidate the impacts of high pH on chlorophyll synthesis and photosynthetic performance in leaves. The chlorophyll score, Fv/Fm, and Pi_ABS exhibited a significant decline in leaves of garden pea under high pH conditions in soil compared to untreated plants (Fig. 2A–2C). Furthermore, Mo, indicative of the rate of closure of reaction centers, demonstrated a significant increase in leaves under high pH conditions relative to controls (Fig. 2D).

Changes in nitrogen fixation genes, and bacterial community in nodules

We counted the number of nodules in the root which exhibited a significant decline in nodules in pea roots in response to high pH (Fig. 3A). Our qPCR experiments demonstrated that the expression of *NifA* and *NifD* significantly declined in nodules due to high pH relative to the plants cultivated with neutral pH. However, the *NifH* showed a significant upregulation in nodules under soil alkalinity compared to controls (Fig. 3B).

We performed Oxford Nanopore Sequencing to analyze the 16S rRNA gene of bacterial communities in nodules collected from pea plants cultivated under control and high pH conditions. Our 16S analysis showed no significant changes in the richness (Chao 1) and diversity (Shannon index) of bacterial communities in root nodules regardless of pH levels in the soil (Fig. 3C-3D). However, the relative abundance analysis showed a significant in *Rhizobium* genera among the top 10 bacterial communities in nodules due to high pH relative to controls (Fig. 3E). At the species level, *Rhizobium indicum*, *Rhizobium leguminosarum*, and *Rhizobium redzepovicii* were the most dominant Rhizobia group among the top 5 bacterial species in pea nodules. Furthermore, all three species showed a significant reduction in the relative abundance of nodules under high pH conditions in contrast to untreated controls (Fig. 3F).

RNA-sequencing analysis for differentially expressed genes (DEGs)

We performed RNA-seq analysis to evaluate the differentially expressed genes in the roots of garden pea exposed to high pH. Principal Component 1 (PC1) accounted for 57.4% of the variability, while PC2 explained 27.4% of the variability for differentially expressed genes in garden pea roots grown under control and high pH conditions (Fig. 4A). The DEGs were plotted in an MA plot, which exhibited a higher number of unchanged DEGs, whereas the number of downregulated DEGs was slightly higher than that of the upregulated DEGs (Fig. 4B). The RNA-seq findings unveiled extensive shifts in the transcriptome of garden pea roots under high pH, with 770 genes exhibiting significant upregulation and 842 genes displaying downregulation (Fig. 4C).

Subsequent functional annotations of these differentially expressed genes (DEGs) were conducted using the EMBL-EBI database specific to garden peas (*Pisum sativum* - Ensembl Genomes 59). This was followed by the generation of heatmaps spotlighting the top 10 upregulated and downregulated (Log2 fold change, $p<0.05$) genes under high pH conditions compared to the control (Fig. 5A-5B, Table 2). Of the upregulated genes, high pH caused a significant upregulation of genes linked to oxidoreductase activity (*Psat1g015240*) and glutathione transferase activity (*Psat1g010280*) in garden peas grown under high pH (Log2 fold change, $p<0.05$) (Fig 5A). Furthermore, genes associated with diverse functions such as metal ion transmembrane transport (*Psat1g011640*), metal ion binding (*Psat6g220320*), peroxidase activity (*Psat1g168160*), zinc ion binding (*Psat1g002000*), Fe ion binding (*Psat5g170360*),

ABC-type transporter activity (*Psat2g009080*), magnesium ion binding (*Psat1g096960*), and cysteine synthase activity (*Psat7g119720*) demonstrated significant upregulation (Log2 fold change, $p<0.05$) under high pH conditions compared to the control (Fig. 5A). In contrast, genes responsible for sulfate transporter activity (*Psat1g040440*), folic acid binding (*Psat5g270080*), photosynthesis (*Psat5g063680*), chlorophyll-binding (*Psat5g200040*), ammonia-lyase activity (*Psat1g046920*), calmodulin binding (*Psat4g048880*), manganese ion binding (*Psat5g094200)*, heme binding (*Psat6g173720*), glutamate-ammonia ligase activity (*Psat1g185960*), and monooxygenase activity (*Psat2g053680*) were significantly downregulated (Log2 fold change, $p<0.05$) under high pH conditions compared to the controls (Fig. 5B, Table 2).

Gene enrichment analysis

Functional enrichment analysis was conducted on both upregulated and downregulated genes using ShinyGO 0.80 bioinformatics tools. Of the biological processes, the most enriched downregulated pathway was photosynthesis, followed by the metabolic process involving porphyrin-containing compounds (Fig. 6A). Similarly, processes such as tetrapyrrole metabolism, tetrapyrrole biosynthesis, and chlorophyll metabolism were also downregulated under high pH conditions. Conversely, certain biological processes like amide biosynthesis, peptide biosynthesis, translation, cellular amide metabolism, peptide metabolism, and organonitrogen compound metabolism were upregulated in garden peas subjected to high pH compared to the control (Fig. 6A). Further, cellular component metabolic processes related to plastids, chloroplasts, cytoplasm, thylakoids, photosynthetic membranes, photosystems, membrane protein complexes, photosystem I, cellular anatomical entities, and membranes were downregulated, while intracellular non-membrane-bounded organelles, ribosomes, intracellular organelles, organelles, intracellular anatomical structures, cellular anatomical entities, cellular components, nucleosomes, and chromatin were upregulated (Fig. 6B). Of the molecular functions of DEGs, genes associated with structural molecular activity, ribosomal structural constituents, chromatin structural constituents, nucleoside phosphate binding, nucleotide binding, small molecule binding, anion binding, purine nucleotide binding, purine ribonucleoside triphosphate binding, and purine ribonucleotide binding were upregulated in garden peas grown under high pH conditions. Conversely, molecular functions related to oxygen binding, carboxylic acid binding, fatty acid binding, metal ion binding, cation binding, amylase binding, and lipid binding were downregulated (Fig. 6C). According to KEGG pathway analysis, pathways including biosynthesis of secondary metabolites, metabolic pathways, porphyrin metabolism, nitrogen metabolism, alanine, aspartate, and glutamate metabolism, biosynthesis of amino acids, sulfur metabolism, photosynthesis, photosynthetic antenna processes, and the MAPK signaling pathway were downregulated during high pH. Conversely, pathways such as valine, leucine, and isoleucine biosynthesis, oxocarboxylic acid metabolism, biosynthesis of amino acids, and ribosomes were upregulated (Fig. 6D).

Changes in root bacterial community due to high pH

The principal coordinates analysis (PCoA) represented different dimensions of variation indicating a clear separation between sample groups (Fig. 7A). In this study, the alpha diversity measures such as Chao 1 (richness), and Simpson index (diversity) of the microbiome community in the roots of garden pea cultivated with or without high pH. We found that microbial communities showed no significant changes in richness, but high pH caused a

significant change in the diversity of bacterial populations due to high pH relative to controls (Fig. 7B). Furthermore, the taxonomic analysis revealed statistically significant ($P<0.05$) differences among several taxonomic classes, orders, and genera of bacterial communities in roots between control and high pH conditions (Fig. 7C-7E). In phyloseq analysis, the relative abundance of *Gammaproteobacteria* and *Actinobacteria* showed a significant increase and decrease in roots in response to high pH compared to controls, respectively (Fig. 7C). However, high pH showed no significant changes in the relative abundance of *Thermolephilia*, *Planctomycetes*, *Ktedonobacteria*, *Clostridia*, *Chloroflexia*, *Bacteroidia*, *Babeliae* and *Alphaproteobacteria* (Fig. 7C). Furthermore, the relative abundance of *Burkholderiales* and *Rhizobiales* showed a significant increase and decrease, respectively, in the roots of garden pea plants exposed to high pH compared to the controls. However, we found no significant difference in the abundance of *Xanthomonadales*, *Streptomycetales*, *Sphinomonadales*, *Pseudomonadales*, *Micrococcales*, *Flavobacteriales* and *Caulobacterales* due to high pH (Fig. 7D). Among the genera, high pH led to a significant increase in the relative abundance of *Variovorax*, *Shinella*, and *Methylophilus* in the roots of garden pea compared to untreated controls (Fig. 7E). Conversely, the relative abundance of *Streptomyces* and *Pararhizobium* significantly decreased under high pH relative to controls. However, no significant changes in abundance were observed for *Pseudomonas*, *Novosphinogobium*, *Mycobacterium*, *Herbaspirillum*, and *Asticcacaulis* (Fig. 7E). In addition, we also determined the siderophore levels in the rhizosphere soil which showed a significant increase due to high pH compared to untreated controls (Fig. 7F).

DISCUSSION

High soil pH presents a significant challenge to agricultural productivity, particularly in legume species like garden pea (Goyal et al. 2021). This study provides novel insights into the transcriptomic profile and microbial dynamics associated with high soil pH, highlighting shifts in key nitrogen-fixing bacteria. Furthermore, the observed shifts in root-associated bacterial communities, characterized by the enrichment of genera underscore the potential of these microbes in coping with micronutrient imbalances under alkaline conditions. This research uncovers extensive transcriptional reprogramming and microbial enrichments in root tissues due to high pH.

Morphological and photosynthetic impairment

The results of our study provide a comprehensive insight into the physiological and molecular responses of garden peas to high pH stress. Plants grown under high pH conditions exhibited stunted growth compared to their control counterparts. This is consistent with previous studies indicating that Fe is crucial for various physiological processes, including chlorophyll synthesis and electron transport in photosynthesis (Aung et al. 2022). We also tested the chlorophyll scoring and OJIP measurements which offered valuable insights into the photosynthetic efficiency and stress responses of the plants (Küpper et al. 2019). The decrease in chlorophyll synthesis and chlorophyll fluorescence parameters underscores the detrimental impact of nutrient imbalance due to high pH on the photosynthetic machinery, leading to reduced energy capture and assimilation, which ultimately manifests as stunted growth and reduced biomass. The significantly lower Fv/Fm ratio highlights the detrimental impact of high pH on the maximal photochemical efficiency of PSII, corroborating findings that micronutrients are essential for optimal photosynthetic performance (Briat et al. 2015). Additionally, the decrease in Pi_ABS in plants exposed to high pH indicates further impairment of the photosynthetic apparatus,

reinforcing the critical role of micronutrients in maintaining photosynthetic efficiency (Ravet and Pilon, 2013).

Rhizobia dynamics in root nodules in response to high pH

We counted the nodules in pea roots that provided important data on the symbiotic relationship between garden peas and nitrogen-fixing bacteria under high pH conditions. Our results showed a significant reduction in nodule number in pea plants exposed to high pH. This finding aligns with earlier reports suggesting that the imbalance of Fe due to high pH in soil, which is vital for the functioning of nitrogenase, the enzyme complex responsible for nitrogen fixation in nodules (Schwember et al. 2019; Brear et al. 2013). The reduced nodule formation could thus be a direct consequence of impaired nitrogen fixation capability by Fe deprivation caused by high pH. Furthermore, the downregulation of *NifA* and *NifD* genes in nodules under high pH conditions supports the idea that Fe is essential for nitrogenase function, with the alkalinity-induced micronutrient imbalance leading to reduced enzyme activity. *NifA* is a transcriptional activator that regulates the expression of other *Nif* genes, including *NifD*, under optimal conditions (Zhang et al. 2023). Thus, lower *NifA* expression under high pH likely leads to a cascade effect, reducing the overall expression of the nitrogenase complex components, and thereby impairing nitrogen fixation. Interestingly, the upregulation of *NifH* under high pH could indicate a compensatory response aimed at maintaining nitrogenase activity despite the overall stress condition and its symbionts' attempt to adjust the nitrogen fixation machinery. This differential gene expression pattern highlights the complex regulatory mechanisms plants and symbiotic bacteria employ to adapt to high pH.

Our 16S rRNA gene analysis revealed no significant changes in the richness and diversity of bacterial communities in root nodules between neutral and high pH conditions. This stability in bacterial community diversity suggests that the overall microbial ecosystem within the nodules remains relatively resilient to alkalinity stress. However, the significant reduction in the relative abundance of *Rhizobium* genera and specific species like *Rhizobium indicum*, *Rhizobium leguminosarum*, and *Rhizobium redzepovicii* under high pH conditions highlights a selective impact of micronutrient availability on key nitrogen-fixing bacteria. These *Rhizobium* species are integral to effective nitrogen fixation, and their reduced abundance could directly contribute to the observed decline in nodule formation and nitrogenase gene expression under high pH in garden pea. Similarly, high pH adversely affected the symbiotic efficiency and nitrogen fixation capacity of *Bradyrhizobium japonicum* and *Mesorhizobium cicero* in legume crops (Merry et al. 2022; Gopalakrishnan et al. 2015). These studies highlight the critical importance of pH for the health and functionality of rhizobial populations in pea nodules. Also, our findings underscore the need for strategies to improve micronutrient availability in soils, which could enhance nitrogen fixation and overall plant productivity in pea crops.

Transcriptional changes in roots

Our RNA-seq analysis has provided a detailed overview of the transcriptional responses of garden pea roots to high pH, revealing significant shifts in gene expression profiles. The principal component analysis indicates that the majority of the variability in gene expression can be attributed to nutrient availability which underscores the profound impact of high pH on the root transcriptome. The identification of 770 upregulated and 842 downregulated genes highlights the extensive transcriptional reorganization occurring in response to high pH. This

also suggests a broad reprogramming of metabolic and physiological pathways in response to Fe stress.

The upregulation of genes associated with oxidoreductase activity (*Psat1g015240*) and glutathione transferase activity (*Psat1g010280*) suggests an enhanced oxidative stress response. These genes likely play crucial roles in mitigating the oxidative damage caused by high pH, which is known to disrupt cellular redox homeostasis (Shee et al. 2022; Tewari et al. 2013). Also, the expression and activity of certain oxidoreductases are often upregulated in plants under high pH (Vigani and Murgia, 2018). For instance, increased activity of enzymes like ferric-chelate reductase is observed in the roots of peas exposed to lime-induced alkaline soil, which aids in the reduction of Fe^{3+} to the more soluble Fe^{2+} form, facilitating its uptake (Kabir et al. 2012). Additionally, other oxidoreductases involved in the ascorbate-glutathione cycle, such as ascorbate peroxidase and glutathione reductase, are crucial for detoxifying ROS produced under Fe stress (Hasanuzzaman et al. 2019). Furthermore, the significant upregulation of genes involved in metal ion transmembrane transport (*Psat1g011640*) and binding (*Psat6g220320*, *Psat1g002000*, *Psat5g170360*) indicates a compensatory mechanism to enhance nutrient uptake and mobilization within the plant. This is consistent with previous findings that plants increase the expression of metal transporter genes to cope with Fe scarcity (Kobayashi and Nishizawa, 2012). The upregulation of ABC-type transporter activity (*Psat2g009080*) and magnesium ion binding (*Psat1g096960*) also points to broader alterations in nutrient transport and homeostasis under high pH conditions. Conversely, the downregulation of genes associated with sulfate transporter activity (*Psat1g040440*), manganese ion binding (*Psat5g094200*), and ammonia-lyase activity (*Psat1g046920*), suggests an impairment of metabolic processes as well as nutrient imbalance and secondary metabolites in peas in response to high pH. The suppression of mineral uptake genes is consistent with the decreased minerals, as Fe and Mn are a critical component of chlorophyll and the photosynthetic electron transport chain (Kroh and Pilon, 2020; Terry and Low, 1982). However, roots are non-photosynthetic organs, so the biological significance of this downregulation may not directly reflect the expression levels of these genes in photosynthetic organs such as leaves. Furthermore, ammonia-lyase enzymes, such as phenylalanine ammonia-lyase (PAL), play critical roles in nitrogen assimilation and the biosynthesis of secondary metabolites, including phenolic compounds (Cass et al. 2015; Kim et al. 2014). Under high pH conditions, the reduced expression of ammonia-lyase genes may indicate that the plant is experiencing a stress-induced imbalance in nitrogen availability, which is consistent with the reduced nodulation and decreased expression of *NifA* and *NifD* genes. High soil pH can limit the solubility and bioavailability of essential nutrients further impairing nitrogen metabolism (Ferrarezi et al. 2022).

Our comprehensive RNA-seq and functional enrichment analysis reveals the intricate molecular and physiological responses of garden pea roots to high pH. The significant differential gene expression, coupled with the detailed functional enrichment analysis, highlights the adaptive strategies employed by plants under nutrient stress. In this analysis, the most enriched downregulated pathway was photosynthesis, which aligns with previous findings indicating that Fe is crucial for chlorophyll synthesis and the photosynthetic electron transport chain (Kroh, & Pilon, 2020). Furthermore, the suppression of tetrapyrrole metabolism, tetrapyrrole biosynthesis, and chlorophyll metabolism pathways suggests a direct consequence of Fe scarcity on the photosynthetic machinery, potentially leading to reduced photosynthetic efficiency and overall

plant growth. Conversely, upregulated processes such as amide biosynthesis, peptide biosynthesis, translation, and various metabolic processes indicate a shift towards maintaining essential cellular functions and protein synthesis under stress conditions. This shift could represent a strategic allocation of resources to sustain fundamental metabolic activities and ensure the survival of garden pea exposed to high pH conditions.

The enrichment analysis of cellular component metabolic processes revealed that plastids, chloroplasts, thylakoids, and related components were significantly downregulated, which is consistent with the reduced photosynthetic activity observed under high pH. The downregulation of photosystems and membrane protein complexes highlights the detrimental effects of abiotic stresses on the photosynthetic apparatus (Nouri et al. 2015). In contrast, the upregulation of intracellular non-membrane-bounded organelles, ribosomes, nucleosomes, and chromatin suggests an increased focus on maintaining ribosomal function and chromatin structure. This may indicate an adaptive response to support protein synthesis and genomic integrity despite the challenging conditions posed by high pH. The upregulation of nucleoside phosphate binding and nucleotide binding activities suggests enhanced nucleic acid metabolism and energy transfer processes, which are critical for maintaining cellular function under stress (Witte and Herde, 2020). Conversely, the downregulation of functions related to oxygen binding, carboxylic acid binding, fatty acid binding, and metal ion binding reflects the broader impact of high pH on metabolic and binding activities. The reduced expression of genes involved in these functions could contribute to compromised metabolic processes and stress responses.

KEGG pathway analysis further corroborates the significant impact of high pH on metabolic and biosynthetic pathways. Downregulated pathways include biosynthesis of secondary metabolites, metabolic pathways, porphyrin metabolism, and photosynthesis-related pathways, underscoring the metabolic constraints imposed by soil alkalinity. The downregulation of the MAPK signaling pathway suggests potential disruptions in signaling mechanisms critical for stress response and adaptation. The MAPK) signaling pathway serves as a significant route through which plants react to abiotic stressors (Sinha et al. 2011). In contrast, upregulated pathways such as valine, leucine, and isoleucine biosynthesis, oxocarboxylic acid metabolism, and ribosomal pathways indicate a strategic focus on amino acid biosynthesis and protein synthesis. These adaptations may help mitigate the adverse effects of high pH by ensuring the continued production of essential proteins and metabolic intermediates in garden pea.

The shift in the bacterial community in the roots under high pH

Our microbiome analysis highlights the shifts in bacterial community structure and composition on the root-associated microbiome in response to high pH in garden pea. The PCoA demonstrated a distinct separation between microbial communities under neutral and high pH conditions, suggesting that the range of pH plays a pivotal role in shaping the root microbiome. While microbial richness remained unaffected by high pH, a significant reduction in microbial diversity was observed. This indicates that although the total number of microbial species was unchanged, High pH selectively influenced the relative abundances of certain bacterial populations, leading to changes in community composition. In general, greater microbial diversity is linked to improved plant fitness and resilience to stress (De Vries et al. 2018). Taxonomic analysis further elucidated the nature of these shifts. The increased relative abundance of *Gammaproteobacteria* and the concurrent decrease in *Actinobacteria* under high

pH conditions suggest that Fe availability differentially affects these microbial groups. *Gammaproteobacteria*, which include several known plant-beneficial genera, may play a compensatory role in alleviating high pH, possibly through mechanisms such as siderophore production or phytohormone synthesis. Gammaproteobacteria have been recognized as an essential part of various plant microbiomes (Erlacher et al. 2014; Fürnkranz et al. 2012). On a finer taxonomic scale, the study revealed significant alterations in the abundance of specific bacterial orders and genera. The increase in *Burkholderiales* and decrease in *Rhizobiales* under high pH point to a reorganization of the root microbial community, where certain taxa may gain competitive advantages or be more resilient under high pH conditions. *Burkholderiales* are often associated with nutrient cycling and stress tolerance (Liu et al. 2021; Enagbonma et al. 2003), suggesting their possible role in mitigating high pH stress in garden pea. In contrast, the decrease in *Rhizobiales*, a group known for its role in nitrogen fixation, may reflect a disruption in plant-microbe symbioses critical for nutrient acquisition under normal conditions. This is consistent with the fact that high pH stress can lead to a disruption in the nodulation process in legumes (Lindström and Mousavi 2020).

At the genus level, the significant increases in *Variovorax*, *Shinella*, and *Methylophilus* suggest that these taxa may have specialized functions in response to high pH, potentially contributing to nutrient acquisition or stress tolerance pathways. Notably, this study represents the first report of these genera's specific roles in the context of soil alkalinity, highlighting their potential importance in the plant microbiome's ability to adapt to high pH stress. Studies showed that *Variovorax boronicumulans* have the potential to help host plants regulate their auxin levels (Sun et al. 2018). *Shinella* can also produce plant growth-promoting substances, such as auxins, which stimulate root development and improve nutrient uptake (Saeed et al. 2021). Auxin facilitates micronutrient uptake specifically Fe in plants by promoting the expression of metal transporter genes and enhancing the growth of root hairs, which increases the plant's ability to absorb micronutrients from the soil (Zhang et al. 2019; Garnica et al. 2018). While the mechanisms are unclear, another report showed that applying *Variovorax* sp. P1R9, either alone or in plant growth-promoting consortia enhanced salt stress tolerance in wheat (Acuña et al. 2024). Additionally, *Methylophilus* is a member of the methylotrophic bacteria that utilize volatile carbon compounds (Meena et al. 2012). Methylotrophs contribute to enhancing crop yield and quality through various functions. They are involved in several biological processes, including nitrogen fixation (Rekadwad, 2014), the production of Fe-chelating compounds (Verma et al. 2015), and the synthesis of hormones (Pattnaik et al. 2017). Thus, the increased abundance of *Variovorax*, *Shinella* and *Methylophilus* observed in this study suggests its potential role in alleviating high pH in garden pea. This is supported by the significant increase in siderophore levels in the rhizosphere under high pH conditions suggesting a robust microbial response aimed at regulating nutrient balance in response to high pH. Siderophores are high-affinity Fe-chelating compounds secreted by microbes to sequester Fe from the environment, particularly under Fe scarcity (Kabir and Bennetzen, 2024; Bhattacharyya and Jha, 2013). Our results indicate that high pH in plants triggers an enhanced microbial siderophore production, likely as a compensatory mechanism to facilitate Fe uptake and alleviate the Fe imbalance caused by high pH. Siderophores are known to play a crucial role in microbial Fe acquisition, and their increased production under Fe-limited conditions has been documented in other legume-microbe interactions (Singh et al. 2022; Samac et al. 2007). This microbial response could potentially aid in mitigating high pH stress in plants by enhancing nutrient availability in the rhizosphere.

Furthermore, certain plant growth promoting genera, such as *Pseudomonas*, *Novosphinogobium*, *Mycobacterium* and *Herbaspirillum*, exhibited no significant changes in abundance. This suggests that these taxa are either insensitive to nutrient availability disrupted due to soil alkalinity or possess adaptive mechanisms that enable their persistence regardless of the pH levels in the soil. These stable populations may serve as key functional components of the core microbiome, maintaining critical processes such as nutrient cycling or pathogen suppression irrespective of pH status. Collectively, these findings underscore the dynamic nature of the root microbiome in response to high pH and highlight key microbial taxa that may contribute to plant adaptation under high pH conditions. Future studies should focus on the functional characterization of these taxa, particularly in the formulation of microbial inoculants or biofertilizers aimed at enhancing plant tolerance to high pH in garden pea and other legume crops.

Conclusion

Our study sheds light on the multifaceted responses of garden peas to high pH stress, encompassing morphological, physiological, molecular, and microbial dynamics. Plants exposed to high pH exhibited stunted growth, impaired photosynthesis, and decreased chlorophyll synthesis, highlighting the critical role of pH level in soil in various physiological processes. Microbial siderophore production increased significantly in the rhizosphere, indicating a compensatory mechanism to enhance nutrient uptake (Fig. 7). Reduced nodule formation and altered gene expression in nodules underscored the impact of high pH on nitrogen fixation. RNA-seq analysis revealed extensive transcriptional reprogramming in roots, with upregulation of oxidative stress response genes and downregulation of photosynthesis-related pathways. Functional enrichment analysis elucidated the adaptive strategies employed by plants, emphasizing resource allocation towards essential cellular functions. KEGG pathway analysis highlighted metabolic constraints imposed by alkali-induced micronutrient scarcity and potential disruptions in stress signaling pathways. Furthermore, we observed that high pH significantly influenced the composition of microbial communities in the roots of garden pea. Specifically, there was a notable increase in the relative abundance of *Variovorax*, *Shinella*, and *Methylophilus* along with stable microbiome (*Pseudomonas*, *Novosphinogobium*, *Mycobacterium* and *Herbaspirillum*) indicating their potential role in adapting to Fe-limited conditions. Overall, our findings emphasize the intricate interplay between soil alkalinity, plant-microbe interactions, and molecular responses, underscoring the importance of addressing high pH for sustainable pea crop productivity.

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Competing interest

We have no conflict of interest.

Author contributions

AT cultivated the plants, measured physiological data, extracted RNA, performed 16S data analysis and prepared the draft manuscript. MRH prepared the 16S library for Nanopore

sequencing. BA helped with 16S data interpretations and revised the manuscript. AHK conceived the idea for the study, interpreted RNA-seq data, supervised the work, and revised the manuscript.

Data availability

Illumina sequencing data was submitted to NCBI under the following BioProject accession numbers: RNA-seq (PRJNA1116995), 16S Nanopore (PRJNA1117024) and 16S Amplicon (PRJNA1169338).

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Table 1. ICP-MS analysis of nutrients in the root and shoot of garden peas grown in control and high-pH soils. Different letters indicate significant differences between the control and high pH conditions at a P <0.05 level. The data represents the means ± SD of three independent biological samples (n = 3).

Elements	Root		Shoot	
	Control	High pH	Control	High pH
Fe (ppm)	384.4±8.7[a]	287.6±8.0[b]	92.9±10.3[a]	58.2±1.2[b]
Zn (ppm)	126.9±15.1[a]	90.2±5.2[b]	52.2±5.9[a]	52.7±4.9[a]
Mn (ppm)	37.7±2.9[a]	22.4±1.9[b]	37.1±2.4[a]	18.3±1.2[b]
S (%)	1.2±0.2[a]	0.7±0.1[b]	0.6±0.1[a]	0.3±0.01[b]
Mg (%)	0.2±0.01[a]	0.2±0.01[a]	0.2±0.01[a]	0.2±0.01[a]
Ca (%)	0.8±0.1[a]	0.7±0.1[a]	1.2±0.01[a]	0.4±0.01[a]

Table 2. The top 10 differentially expressed genes in roots of garden pea exposed to high pH relative to controls. Values marked with an asterisk are significant (> Log2 fold, < P value 0.05).

Upregulated genes	High pH vs Control (fold change)
Psat1g015240 (oxidoreductase activity)	5.1
Psat1g010280 (glutathione transferase activity)	4.6
Psat1g011640 (metal ion transmembrane transporter)	3.8

Psat6g220320 (metal ion binding)	4.4
Psat1g168160 (peroxidase activity)	4.2
Psat1g002000 (zinc ion binding)	3.0
Psat5g170360 (Fe ion binding)	3.2
Psat2g009080 (ABC−type transporter activity)	2.3
Psat1g096960 (magnesium ion binding)	2.3
Psat7g119720 (cysteine synthase activity)	2.9
Downregulated genes	
Psat1g040440 (sulfate transporter activity)	-2.7
Psat5g270080 (folic acid binding)	-2.1
Psat5g063680 (photosynthesis)	-2.0
Psat5g200040 (chlorophyll binding)	-2.1
Psat1g046920 (ammonia−lyase activity)	-3.0
Psat4g048880 (calmodulin binding)	-2.0
Psat5g094200 (manganese ion binding)	-2.0
Psat6g173720 (heme binding)	-2.9
Psat1g185960 (glutamate−ammonia ligase activity)	-2.1
Psat2g053680 (monooxygenase activity)	-2.0

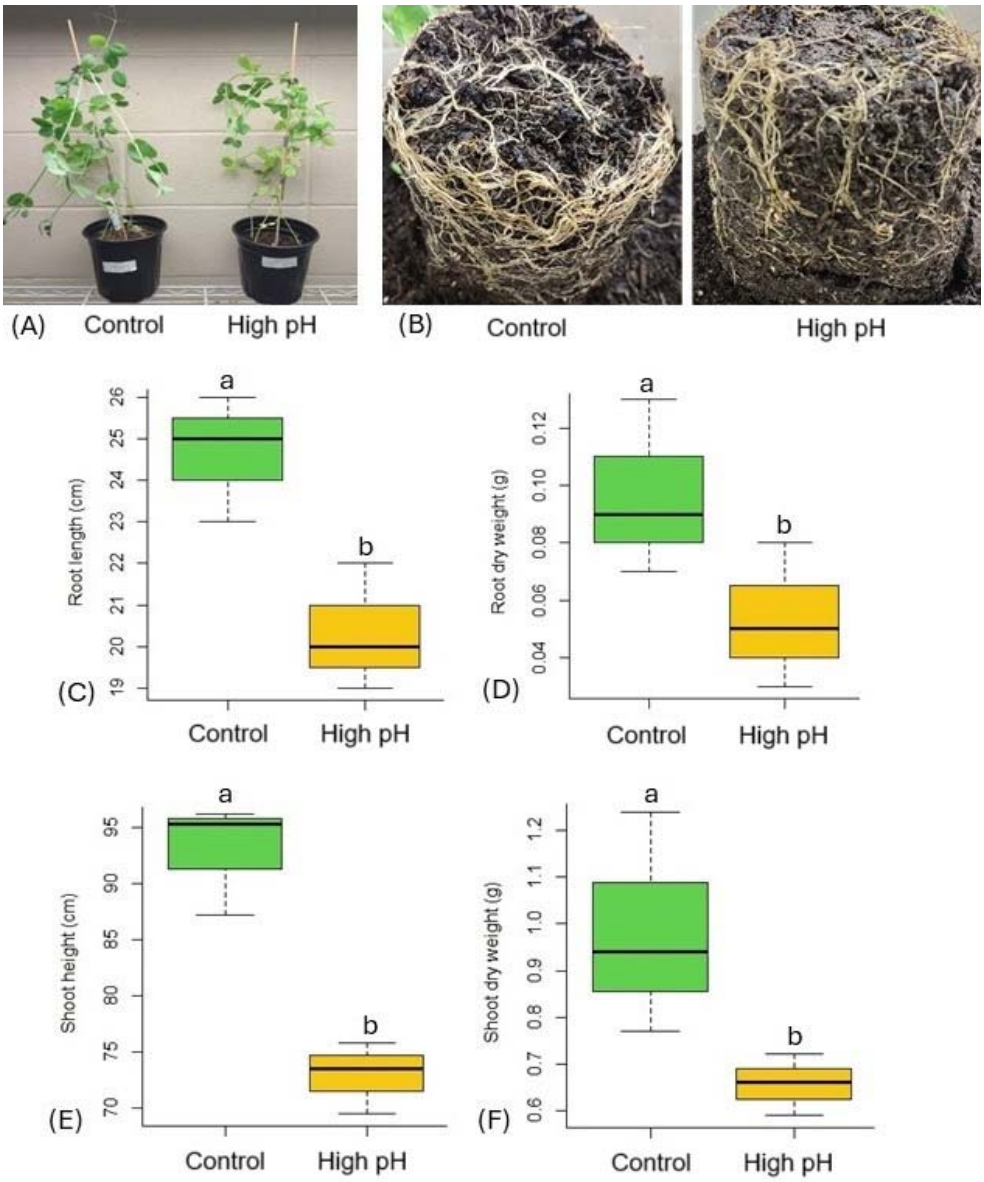

Fig 1. Plant phenotype and morphology of garden peas grown in control and high pH conditions: aboveground phenotype (A), root length (B), root dry weight (C), shoot length (D), and shoot dry weight (E) after five weeks of transplantation. Different letters indicate significant differences between the control and high pH conditions at a $P < 0.05$ level. The data represents the means ± SD of three independent biological samples ($n = 3$).

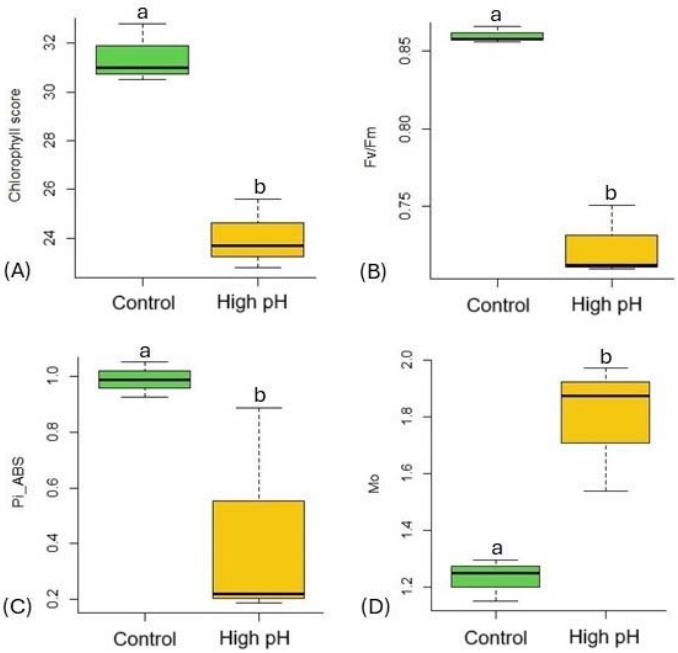

Fig 2. Chlorophyll score (A), Fv/Fm (maximal quantum yield of PS II (B), Pi_ABS (photosynthesis performance index) (C) and Mo (approximated initial slope of (in ms^{-1}) of the fluorescent transient) (D) in leaves of garden pea cultivated in control and high pH conditions. Different letters indicate significant differences between the control and high pH conditions at a P <0.05 level. The data represents the means ± SD of three independent biological samples (*n* = 3).

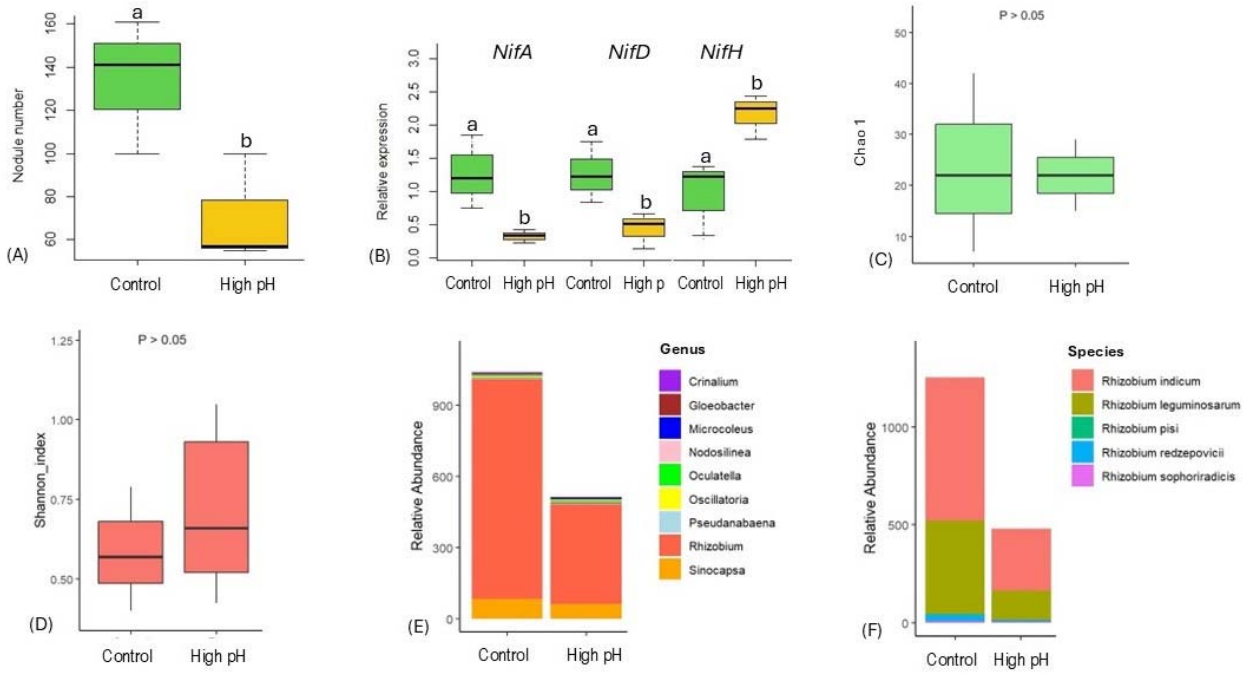

Fig 3. Measurement of root nodule numbers (A), relative expression of *nif* genes (B): *nifA*, *nifD*, and *nifH*, Chao 1 index (C), Shannon-index (D) for bacterial communities of root nodules, relative abundance of top 10 bacterial genus (E) and relative abundance of top 5 Rhizobia species (F) in root nodules of garden peas cultivated in control and high pH conditions.

Different letters indicate significant differences between the control and high pH conditions at a P <0.05 level. The data represents the means ± SD of three independent biological samples (*n* = 3).

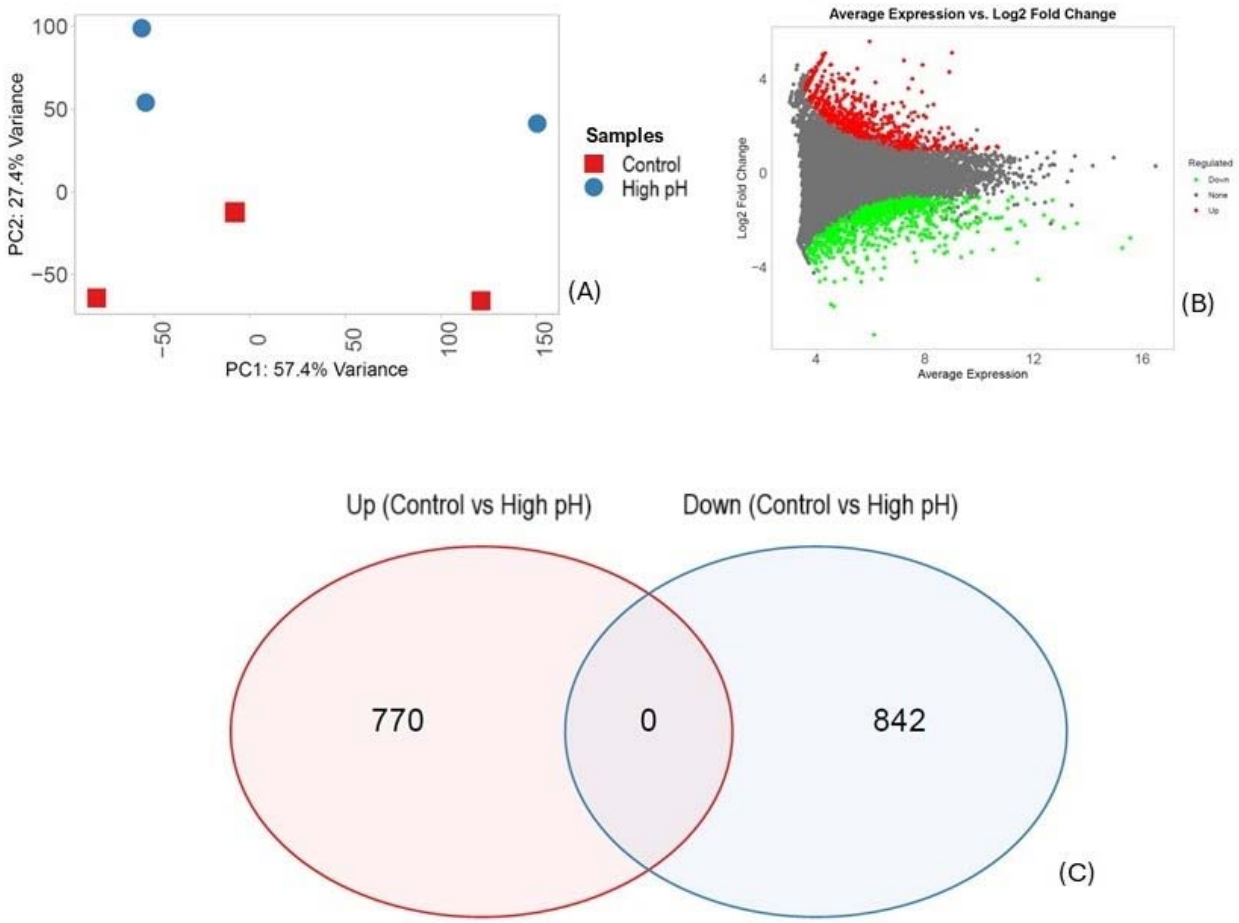

Fig. 4. Analysis of RNA-sequencing data for differentially expressed genes (DEGs): (A) principal component analysis of DEGs, (B) MA plot of genes for Fe- vs. control (>Log2 fold, p<0.05) and (C) vein diagram of upregulated and downregulated genes of garden peas grown in High pH vs. control.

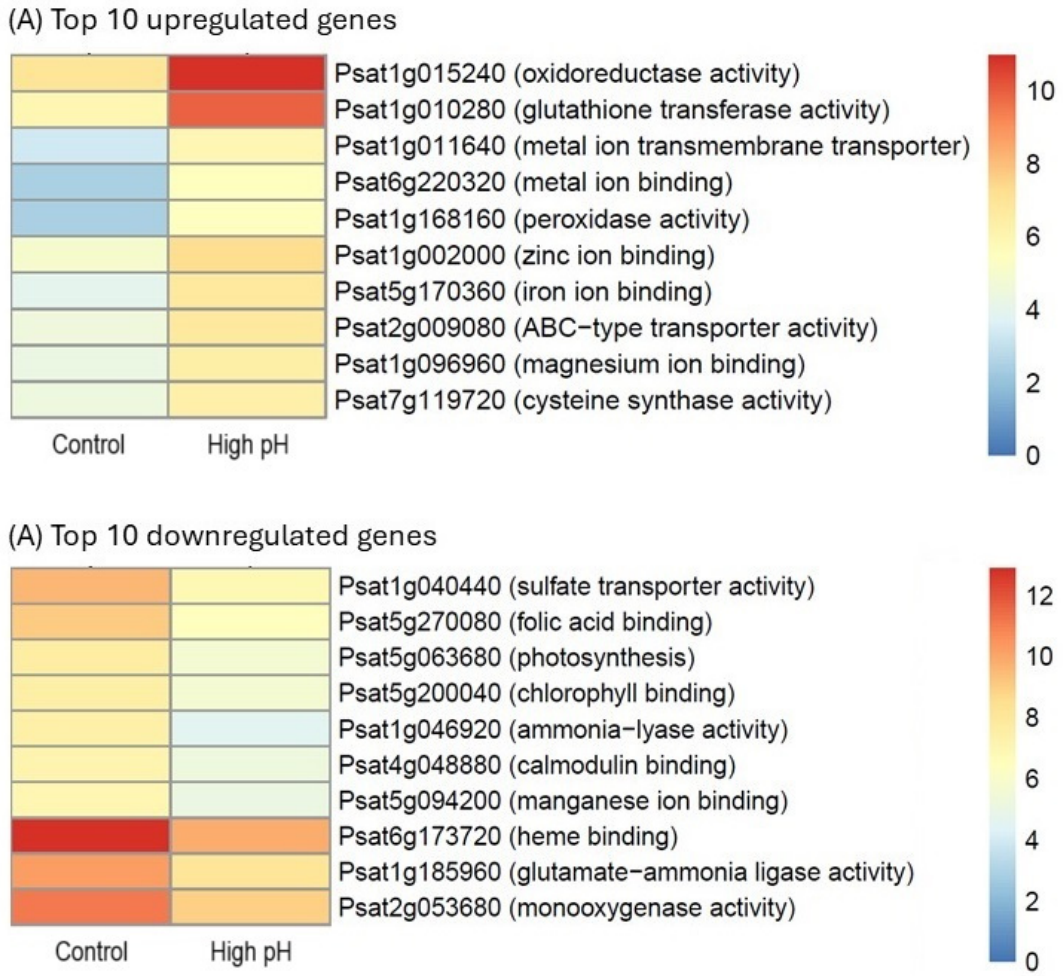

Fig 5. Heatmaps for top 10 upregulated genes (A) and for top 10 downregulated genes (B) of differentially expressed genes (DEGs) in roots of garden peas grown in High pH vs control.

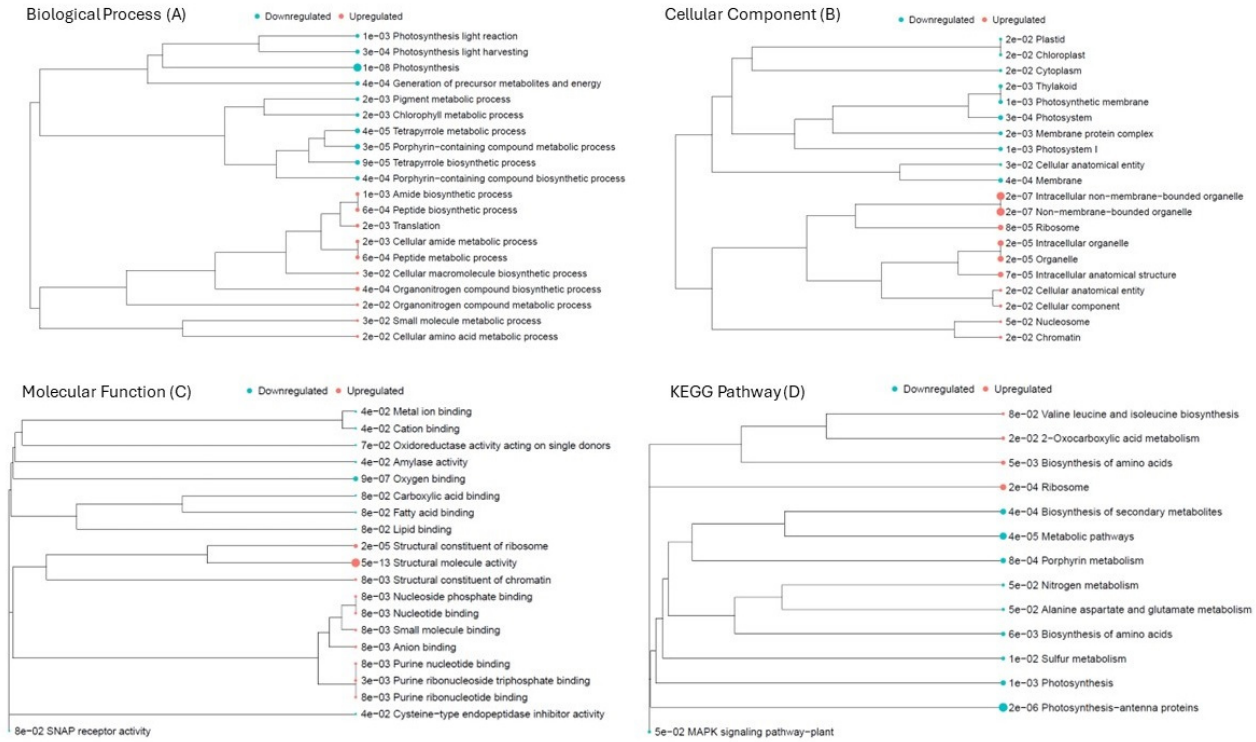

Fig 6. Gene enrichment analysis for the upregulated and downregulated differentially expressed genes (DEGs) of garden peas roots cultivated in high pH and neutral pH conditions: (A) enrichment analysis of upregulated and downregulated genes of roots of garden peas grown in Fe- and control conditions for biological process, (B) enrichment analysis of upregulated and downregulated genes of roots of garden peas grown in high pH and control conditions for cellular components, (C) enrichment analysis of upregulated and downregulated genes of roots of garden peas grown in Fe- and control conditions for molecular functions, and (D) KEGG pathway analysis upregulated and downregulated genes of roots of garden peas grown in high pH and control conditions.

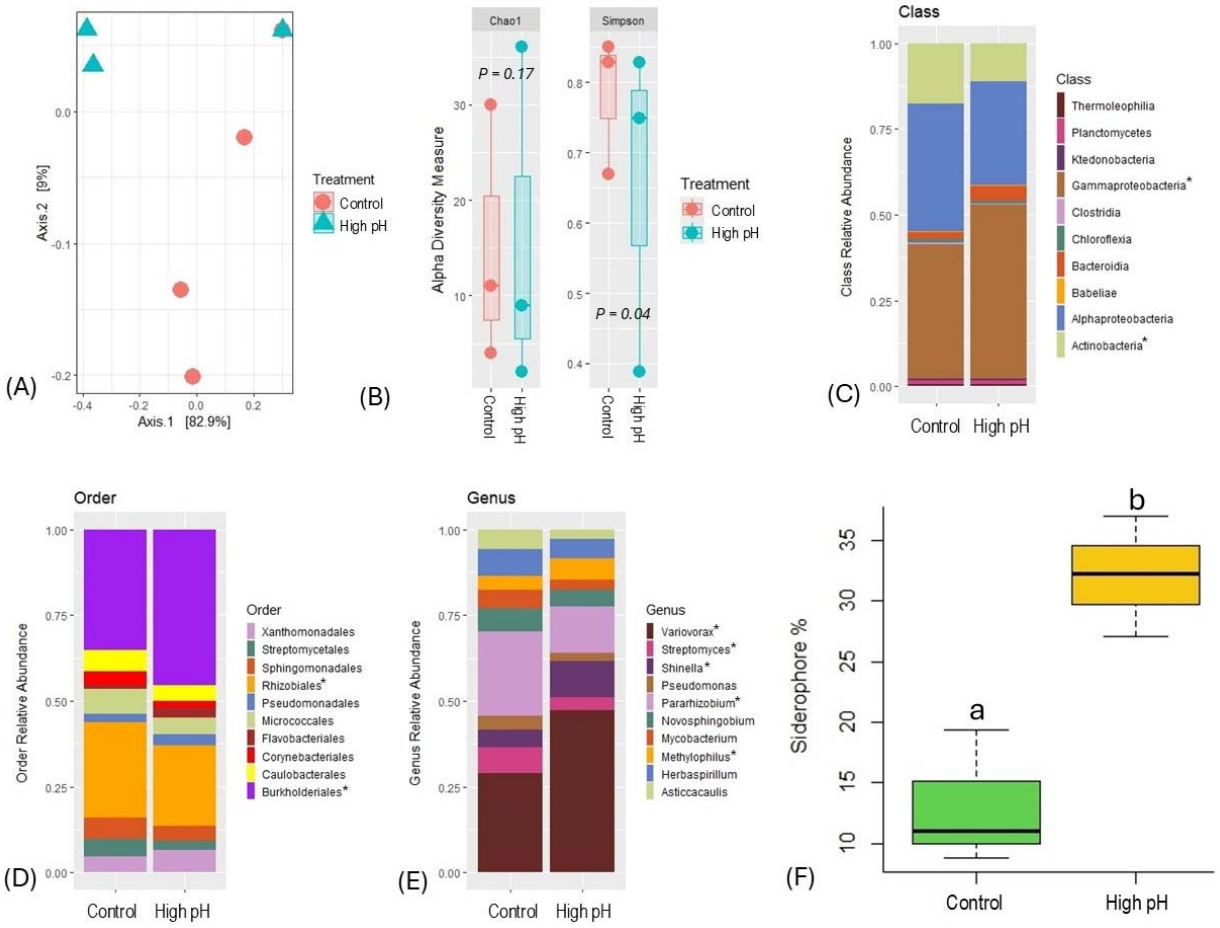

Fig 7. Principal coordinate analysis (PCoA) showing the community compositions assignments of bacterial 16S genes (A), alpha diversity measures (B): Chao 1, and Simpson index, class-level relative abundance of bacterial taxa (C), order-level relative abundance of bacterial taxa (D) and genus-level relative abundance of bacterial taxa (E) in the roots and rhizosphere siderophore (F) of pea plants cultivated in the absence (control) or presence of high pH. Taxa marked with asterisks indicate significant differences between the two growth conditions.

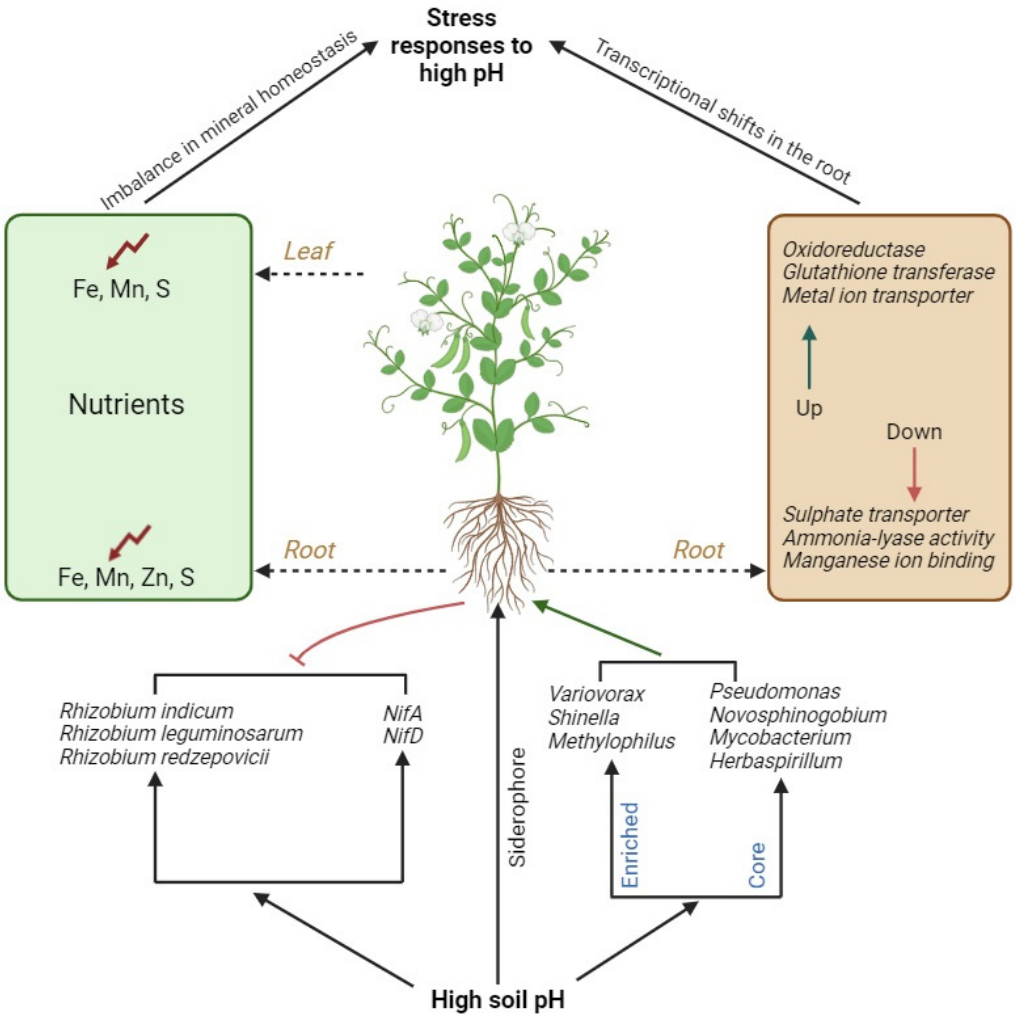

Fig. 8. Model of interaction between garden pea and microbiome under high pH in soil. Soil alkalinity induces rhizosphere siderophore and suppresses Rhizobia (*Rhizobium indicum*, *Rhizobium leguminosarum*, *Rhizobium redzepovicii*) as well as the expression of *NifA* and *NifD* in nodules. High pH led to the enrichment of *Variovorax*, *Shinella*, and *Methylophilus*, while several core microbiomes, including *Pseudomonas*, *Novosphingobium*, *Mycobacterium*, and *Herbaspirillum*, remained stable in the roots. High pH in soil caused a significant decrease in mineral levels in both root and shoot. Among the differentially expressed genes, those encoding oxidoreductases, glutathione transferases, and metal ion transporters showed significant upregulation, while genes associated with sulfate transporters, ammonia-lyase activity, and manganese ion binding were downregulated under high pH conditions.