



RESEARCH ARTICLE OPEN ACCESS

Trichoderma Mobilizes Phosphorus and Cobalt While Excluding Sodium in the Foliar Ionome of High-Andean Grasses Enriched With Biochar

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Received: 19 March 2026 | **Revised:** 22 June 2026 | **Accepted:** 25 June 2026

Funding: Instituto Nacional de Innovación Agraria, Grant/Award Number: CUI Project No. 2487112 - INIA

Keywords: high-Andean grasslands | high-Andean soils | leaf ionomics | mineral nutrients | multivariate analysis

ABSTRACT

Acidic high-Andean soils constrain grassland productivity by limiting nutrient availability and altering plant ionic balance. Biochar inoculated with plant growth-promoting microorganisms has emerged as a promising strategy to mitigate these constraints; however, the extent to which microbial identity modulates plant nutrient acquisition remains poorly understood. This study evaluated the effects of *Trichoderma* sp., *Bacillus* sp., and *Pseudomonas* sp. as inoculants of guinea pig manure-derived biochar on soil pH and the foliar ionome of *Carex* sp., *Dactylis glomerata*, *Festuca dolichophylla*, and *Lolium perenne* in a high-Andean Andisol (4150 m a.s.l.). A randomised complete block design (4 × 4 factorial, three replicates) was used. Biochar (20 t ha⁻¹) was applied at establishment, and microbial inoculants (5 L ha⁻¹) were applied at sowing and during crop development. Foliar elemental concentrations were determined by MP-AES and analysed using ANOVA and multivariate approaches. Biochar significantly increased soil pH ($p < 0.001$). Ionomic responses depended on microbial identity: *Trichoderma* sp. enhanced P and Co accumulation while reducing Na ($p < 0.05$), indicating coordinated nutrient regulation. Micronutrients (Fe, Mn, Cu) were primarily species-driven. These findings demonstrate that microbially functionalized biochar can selectively reconfigure plant ionomes, supporting microbiome-assisted strategies to improve nutrient efficiency and resilience in acidic agroecosystems.

1 | Introduction

High-mountain Andean ecosystems in Peru, located at elevations above 4000 m a.s.l., constitute critical environments that support livestock production systems based on native grasslands and introduced forage species. These puna ecosystems are essential for regional food security; however, the productivity and nutritional quality of dominant taxa such as *Festuca dolichophylla*, *Lolium perenne*, *Dactylis glomerata*, and forage species of

the genus *Carex* are constrained by severe biogeochemical limitations. Extreme edaphic acidity (pH < 4.8), low base saturation, and limited availability of phosphorus (P) and potassium (K) are characteristic features of high-altitude Andisols and Entisols. These soils are frequently subjected to intense water erosion and prolonged overgrazing. Such degradative processes accelerate the depletion of soil organic carbon and reduce the cation exchange capacity (CEC), thereby compromising soil

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resilience under changing climatic conditions (Poblete-Grant et al. 2021; Oñate-Valdivieso, Oñate-Paladines, and Díaz 2024).

Soil pH acts as a key regulator of nutrient speciation and bio-availability. In strongly acidic environments of the high Andes, increased Al^{3+} and Fe^{3+} activity promotes phosphate precipitation, thereby reducing the labile P fraction, while limited deprotonation of colloidal surfaces constrains the retention of basic cations (Ca^{2+} , Mg^{2+} and K^{+}) (Yang et al. 2024; Muindi 2019). At high elevations, aluminium's phytotoxicity not only restricts root elongation but also compromises plasma membrane integrity and the efficiency of electrochemical gradient-dependent transporters (H^{+} -ATPase), ultimately limiting water and mineral uptake (Wei et al. 2020; Zhang et al. 2017). Consequently, these edaphic disturbances are reflected in the configuration of the foliar ionome, a multi-element profile that integrates the complex interactions among rhizosphere availability, root system architecture, and physiological homeostasis (Watanabe et al. 2015).

As a restoration strategy, biochar has emerged as a recalcitrant amendment with a high mitigation potential. Biochar derived from animal residues, particularly guinea pig (*Cavia porcellus*) manure, exhibits significantly higher ash content and greater acid-neutralising capacity than conventional lignocellulosic biochar (Ccopi et al. 2025; Murtaza et al. 2024; Enders et al. 2012). Their porous microstructure not only increases the specific surface area available for ionic adsorption but also functions as a thermal and biological micro refuge, protecting soil microbiota against recurrent frost events and intense solar radiation typical of ecosystems located above 4000 m a.s.l. (Bong, Lim, and Lee 2020; Chen et al. 2025).

Recently, the functionalization of biochar through inoculation with Plant Growth-Promoting Microorganisms (PGPM), including genera such as *Bacillus*, *Pseudomonas* and *Trichoderma*, has been proposed as a biotechnological frontier. These microorganisms enhance soil fertility by promoting phosphate solubilisation and producing siderophores and organic acids, thereby facilitating plant nutrition under multivariable stress conditions (Kamyab et al. 2025; Yao et al. 2023).

Despite its potential, a significant knowledge gap persists regarding the synergistic effects of these microbial conditioners at extreme elevations, where the concurrent dynamics of macro-, micro-, and trace elements remain poorly understood. In particular, further evidence is required to elucidate how such amendments modulate the foliar ionome in a species-dependent manner under soils affected by active erosional processes. Therefore, the objective of this study was to evaluate the impact of a guinea pig manure-derived biochar microbial conditioner on edaphic pH and on the concentrations of foliar nutrients and trace elements in high-Andean forage species, comparing the effects of inoculation with *Bacillus* sp., *Pseudomonas* sp. and *Trichoderma* sp. We hypothesised that the application of this functionalized biochar mitigates soil acidity irrespective of the microbial inoculant, whereas the reconfiguration of the foliar ionome is dependent on microbial identity.

2 | Materials and Methods

2.1 | Study Area and Experimental Conditions

The experiment was conducted during the 2023–2024 cropping season in the high-Andean zone of the Pasco region, Peru, specifically in the district of Simón Bolívar, Pasco Province and Region, at an altitude of 4150 m a.s.l. The experimental site is located at the geographical coordinates $10^{\circ}50' \text{ S}$ and $76^{\circ}16' \text{ W}$, within the natural Puna region. The climate corresponds to an alpine tundra type (ET) according to the Köppen–Geiger classification (Beck et al. 2018). During the experimental period, air temperature ranged from -0.4°C to 16.0°C in the rainy season and from -8.2°C to 15.5°C in the dry season. Mean relative humidity was 78.1%, and the cumulative annual precipitation reached 802.4 mm. Climatic data were obtained from the Cerro de Pasco meteorological station operated by the National Service of Meteorology and Hydrology of Peru (SENAMHI 2026).

The soil at the experimental site was characterised by a loamy texture and a moderately acidic reaction (pH 5.0). Initial physicochemical properties were determined using standardised protocols. Soil pH was measured following EPA method 9045D (USEPA 2004), whereas soil texture and organic matter content (9.20%) were analysed in accordance with NOM-021-RECNAT-2000, using the Bouyoucos and Walkley–Black methods, respectively. Available phosphorus (13.70 mg kg^{-1}) was determined using the Bray and Kurtz 1 method, and available potassium (89.90 mg kg^{-1}) was quantified by inductively coupled plasma optical emission spectrometry (ICP-OES) following EPA method 6010D. Exchangeable acidity ($1.66 \text{ cmolc kg}^{-1}$) and exchangeable aluminium ($0.09 \text{ cmolc kg}^{-1}$) were determined by potassium chloride extraction (AS-33) (SEMARNAT 2002; Sales-Dávila et al. 2024). Although the soil exhibited moderately acidic conditions, the relatively high organic matter content may have contributed to maintaining moderate levels of available phosphorus. Organic matter can reduce phosphorus fixation by forming complexes with aluminium and iron, thereby increasing P availability in acidic soils (Johan et al. 2021). This mechanism may explain the observed phosphorus levels despite the acidic soil reaction. Initial soil nitrogen content was not determined, which represents a limitation for a more comprehensive assessment of soil fertility and nutrient dynamics. These edaphic conditions, representative of degraded high-Andean soils of the central Peruvian highlands where acidity constrains nutrient bioavailability, provided the basis for evaluating the mitigation potential of guinea pig manure-derived biochar. Specifically, its effectiveness was assessed when functionalized with plant growth-promoting rhizobacteria (PGPR) as a strategy to restore soil fertility in these fragile ecosystems.

2.2 | Experimental Design and Factors

The experiment was conducted under field conditions in the high-Andean zone of Junín, Peru. A 4×4 factorial arrangement was established within a randomised complete block design (RCBD), with three replications, resulting in a total of 48 experimental units. Blocks were arranged to account for spatial variability across the field and to reduce experimental error associated with potential edaphic or micro-environmental gradients. Each experimental unit consisted of a 4 m^2 plot

(2 × 2 m), delimited to ensure soil conditioning independence and to minimise border effects. Plots within each block were separated by 1 m, and adjacent blocks were also spaced 1 m apart. These buffer zones were established to minimise potential lateral movement of nutrients and microbial interactions between soil conditioning.

The experiment followed a 4 × 4 factorial design with two factors. The first factor was forage species, comprising four levels: *Festuca dolichophylla*, *Carex* sp., *Lolium perenne* and *Dactylis glomerata*. The second factor was soil conditioning, with four levels: (i) control (no amendment or microbial inoculation), and (ii–iv) guinea pig manure-derived biochar inoculated with *Bacillus* sp., *Pseudomonas* sp., and *Trichoderma* sp., respectively. Biochar was manually incorporated into the upper 15–20 cm of the soil at a rate of 20 t ha⁻¹ prior to pasture establishment. Microbial inoculants were applied at sowing and subsequently re-applied at 30, 90, and 120 days after sowing (25 mL of culture per liter of water; equivalent dose of 5 L ha⁻¹ per application), resulting in a cumulative input of 20 L ha⁻¹ of microbial suspension throughout the crop cycle.

Agronomic management was standardised across all experimental plots to minimise external sources of variation and to ensure that observed differences in soil pH and foliar ionic composition were attributable to the experimental factors and their interaction. All plots were managed under rainfed conditions, with no supplemental irrigation applied. Sowing procedures and plant density were kept consistent across all plots. Manual weed control was performed at regular intervals, and no fertilisers or additional agrochemical inputs were applied throughout the experimental period. No pest or disease control measures were required during the study.

Despite these controls, some methodological limitations should be acknowledged. The relatively small plot size may introduce edge effects, even with the implemented buffer zones. In addition, the biochar application rate used in this study may limit direct extrapolation to large-scale field conditions. Furthermore, the absence of temporal measurements restricts the assessment of dynamic changes in soil and plant responses over time.

2.3 | Biochar Production and Microbial Inoculation

Biochar was produced from guinea-pig manure (*Cavia porcellus*), which was sieved and air-dried until a moisture content below 20% was achieved. Conversion was carried out through slow pyrolysis in a fixed-bed reactor at 400°C for 3–4 h (Yang et al. 2016; Yu et al. 2019). The biochar used in this study is essentially the same material previously characterised under identical feedstock and pyrolysis conditions in a prior study by our research group. That study reported an alkaline pH (9.17), high electrical conductivity (17.28 dS m⁻¹), and elevated CEC (48.8 meq. 100 g⁻¹), as well as substantial organic matter content (62%) and approximately 1% nitrogen. It also contained relevant macro- and micronutrients (Ca, Mg, K, Fe, Zn, and Mn), supporting its potential to influence soil chemical processes. Proximate analysis of the feedstock indicated high

volatile matter (65.4%), ash content (34.6%), and fixed carbon (37.9%), reflecting its suitability for biochar production. FTIR analysis from the same study identified aromatic and oxygen-containing functional groups (850–897 cm⁻¹), which may contribute to the reactivity of the resulting biochar (Solórzano et al. 2026). However, specific surface area and detailed functional group characterisation of the biochar were not determined, which may limit a more comprehensive mechanistic interpretation.

For the inoculation phase, strains of *Bacillus subtilis* and *Pseudomonas putida* (previously identified by 16S rRNA sequencing), as well as *Trichoderma* sp., were used. The bacterial strains were cultured in nutrient broth at 28°C until reaching a cell density of 1 × 10⁹ UFC mL⁻¹, and were subsequently diluted to 1% (v/v) for field application (Solórzano-Acosta and Quispe 2024). *Trichoderma* sp., in turn, was prepared as a spore suspension derived from colonised maize substrate (1 × 10¹² conidios kg⁻¹) at a concentration of 40 g L⁻¹ (Huasasquiche et al. 2024). Biochar inoculation was performed by manual spraying of the microbial suspensions onto the amendment, ensuring homogeneous distribution and effective adsorption of the microbial consortia prior to their incorporation into the 4-m² experimental plots. This procedure enabled biochar to function as both a physical carrier and a carbon source for the microorganisms, thereby enhancing their plant growth-promoting effects (PGPR) under the climatic conditions of the central Andean highlands.

2.4 | Soil Sampling and Laboratory Analysis

At the end of the experimental period, composite soil samples were collected from each experimental unit from the surface layer (0–20 cm), corresponding to the zone of highest root activity in high-Andean grasslands. The samples were homogenised, air-dried at room temperature, and sieved through a 2-mm mesh to ensure uniformity in subsequent analytical determinations. Soil pH was determined using the potentiometric method following EPA standard 9045D (USEPA 2004). A soil: water suspension at a 1:1 ratio (w/v) was prepared using deionised water, and measurements were performed with a previously calibrated digital pH metre. This procedure enabled accurate quantification of active soil acidity and the changes induced by the incorporation of guinea-pig manure biochar (*Cavia porcellus*) functionalized with microbial consortia.

Soil phosphorus and other nutrient parameters were not determined at the end of the experiment, as the study was specifically designed to focus on soil pH dynamics and plant ionic responses. This represents a limitation for fully assessing soil conditioning effects on soil fertility.

2.5 | Plant Sampling and Foliar Elemental Analysis

At harvest, representative foliar samples of each taxon were collected from the 48 experimental units. Plant material was washed with distilled water to remove surface impurities and subsequently oven-dried in a forced-air circulation system at 65°C until constant weight was achieved. The dried samples

were then ground using a stainless-steel blade mill to pass through a 1-mm mesh. For ionic determination, plant tissue was subjected to wet acid digestion using high-purity concentrated HNO_3 in a digestion block. Concentrations of macronutrients (Ca, Mg, P, K), micronutrients (Fe, Mn, Cu, Mo), and trace elements (Ba, Co, Ni, Sr, Na) were quantified by microwave plasma-atomic emission spectrometry (MP-AES) (Balaram 2020). To ensure analytical accuracy and reproducibility, reagent blanks and certified reference materials were processed within each analytical batch. Results were expressed on a dry matter basis as mg kg^{-1} .

2.6 | Statistical Analysis

Data were analysed using R software version 4.4.2 (R Core Team 2024). To evaluate the effects of forage species, soil conditioning, and their interaction on soil pH and foliar mineral concentrations, a two-way factorial analysis of variance (ANOVA) was conducted under an RCBD. Assumptions of normality and homogeneity of variances were verified using the Shapiro-Wilk and Levene tests, respectively. When significant differences were detected ($p < 0.05$), multiple mean comparisons were performed using Tukey's honestly significant difference (HSD) test. In the presence of significant interactions, simple comparisons within each species were carried out using estimated marginal means. The relationships between soil and foliar variables were assessed using Pearson correlation coefficients (r). Multivariate analysis was performed through Principal Component Analysis (PCA) on previously standardised variables (mean = 0, standard deviation = 1). Results were visualised using graphical representations generated in R.

3 | Results

3.1 | Effect of Forage Species and Inoculated Biochar on Soil pH

The analysis of variance revealed a significant effect of the inoculated biochar soil conditioning on soil pH ($F = 8.594$, $p < 0.001$), whereas the forage species factor did not show a significant effect ($p = 0.085$). Likewise, no significant interaction between forage species and soil conditioning was detected ($p = 0.875$) (Table 1), indicating that the pH response was consistent across the evaluated forage species. The block effect was not significant ($p = 0.395$), suggesting that spatial variability was adequately controlled by the experimental design.

The analysis of variance indicated that soil conditioning significantly affected soil pH ($p < 0.001$), whereas forage species

had no significant effect ($p = 0.085$). The interaction between both factors was not significant ($p = 0.875$) (Table 1).

Consistent with these results, Figure 1 illustrates the magnitude of this change: the incorporation of inoculated guinea-pig manure biochar (*Cavia porcellus*) shifted soil pH from a strongly acidic condition in the control (~ 5.2) toward slightly acidic or near-neutral ranges (~ 5.9 – 6.2).

According to Tukey's mean comparison test ($p < 0.05$), the three inoculated soil conditioning (*Bacillus* sp., *Pseudomonas* sp. and *Trichoderma* sp.) were statistically grouped together (letter "b"), consistently outperforming the control group (letter "a"). This pattern confirms that the neutralisation potential is associated with the intrinsic alkalising properties of guinea-pig-derived biochar, which acts as an effective amendment for restoring chemical balance in degraded Andisols and Entisols of the central highlands.

3.2 | Differential Accumulation of Foliar Macronutrients in Response to Inoculated Biochar Across Forage Species

The analysis of variance showed that forage species significantly influenced foliar concentrations of Ca, Mg, P, and K ($p < 0.001$ in all cases; Table 2). Soil conditioning had a significant main effect only on foliar phosphorus ($F = 26.05$; $p < 0.001$). Significant forage species $\times$ soil conditioning interactions were observed for Mg, P and K ($p < 0.05$), indicating that the response to inoculation depended on forage species identity.

For Mg (Figure 2A), although the main soil conditioning effect was not significant in the ANOVA, the significant forage species $\times$ soil conditioning interaction was expressed through contrasting responses among forage species. *Dactylis glomerata* exhibited significant increases under inoculated biochar application, particularly with *Pseudomonas* sp., whereas *Festuca dolichophylla* showed minimum values in the control and higher concentrations under inoculated soil conditioning, confirming that the response was genotype-dependent.

For P (Figure 2B), the significant soil conditioning effect detected in the ANOVA was reflected in consistent increases under inoculated biochar compared with the control, particularly in *Dactylis glomerata*, *Festuca dolichophylla* and *Lolium perenne*. The significant interaction indicates that the magnitude of the increase varied among forage species, being more pronounced under *Trichoderma* sp. inoculation in some of them. For K

TABLE 1 | Two-way ANOVA assessing the effects of forage species and soil conditioning on soil pH.

Source of variation	df	Sum of squares	Mean square	F	p
Block	2	0.386	0.1931	0.957	0.395
Forage species	3	1.466	0.4885	2.421	0.085
Soil conditioning	3	5.202	1.7341	8.594	< 0.001***
Forage species $\times$ Soil conditioning	9	0.875	0.0972	0.482	0.875
Residuals	30	6.054	0.2018	—	—

Note: Values correspond to the F statistics (F) and associated probability values (p) obtained from a two-way ANOVA including block (random effect), forage species and soil conditioning (fixed factors), and their interaction. Significance levels are indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

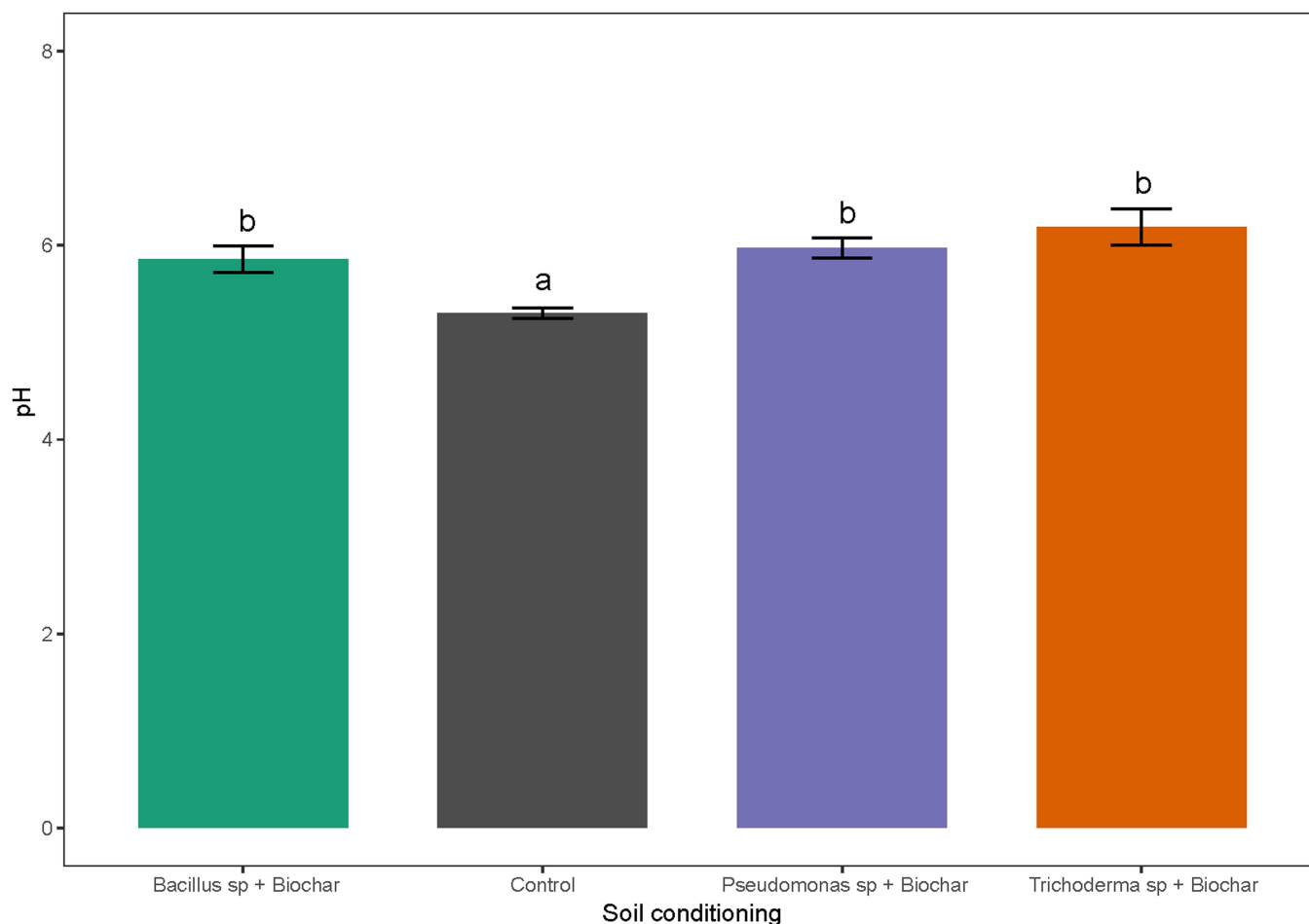


FIGURE 1 | Effects of soil conditioning on soil pH in high-Andean forage species. Bars represent means $\pm$ standard error. Different letters indicate significant differences among soil conditioning combinations according to Tukey's HSD test ($p < 0.05$).

TABLE 2 | Two-way ANOVA results for foliar concentrations of macronutrients (Ca, Mg, P, and K) as affected by forage species and soil conditioning.

Source of variation	df	Ca (F)	Ca (p)	Mg (F)	Mg (p)	P (F)	P (p)	K (F)	K (p)
Block	2	7.04**	0.003	2.88	0.072	1.14	0.334	2.7	0.084
Forage species	3	14.28	< 0.001***	38.37	< 0.001***	33.04	< 0.001***	63.97	< 0.001***
Soil conditioning	3	2.21	0.108	0.79	0.51	26.05	< 0.001***	0.82	0.496
Forage species $\times$ Soil conditioning	9	1.11	0.385	5.97	< 0.001***	2.5	0.029*	5.67	< 0.001***
Residuals	30	—	—	—	—	—	—	—	—

Note: Values correspond to F statistics (F) and associated probability values (p) obtained from a two-way ANOVA including block (random effect), forage species and soil conditioning (fixed factors), and their interaction. Significance levels are indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

(Figure 2C), the absence of a main soil conditioning effect, together with the presence of a significant interaction, confirms that the differences observed in Figure 2 were species-specific. *Dactylis glomerata* and *Lolium perenne* showed positive responses to the inoculated soil conditioning, whereas in *Carex* sp., the differences were less marked.

3.3 | Interspecific Variation in Foliar Concentrations of the Micronutrients Fe, Mn, and Cu

The analysis of variance indicated that forage species significantly influenced foliar concentrations of Fe ($F = 3.58$;

$p = 0.025$), Mn ($F = 13.51$; $p < 0.001$), and Cu ($F = 5.61$; $p = 0.004$), whereas no significant differences were detected for Mo ($p = 0.907$; Table 3). In contrast, neither the inoculated soil conditioning nor the forage species $\times$ soil conditioning interaction showed significant effects on any of the evaluated micronutrients ($p > 0.05$), indicating that their concentrations were primarily determined by forage species identity.

The nature of these interspecific differences is illustrated in Figure 3. For iron (Figure 3A), *Festuca dolichophylla* showed the highest concentrations, differing significantly from *Lolium perenne*, whereas *Carex* sp. and *Dactylis glomerata* exhibited intermediate values. In the case of manganese (Figure 3B),

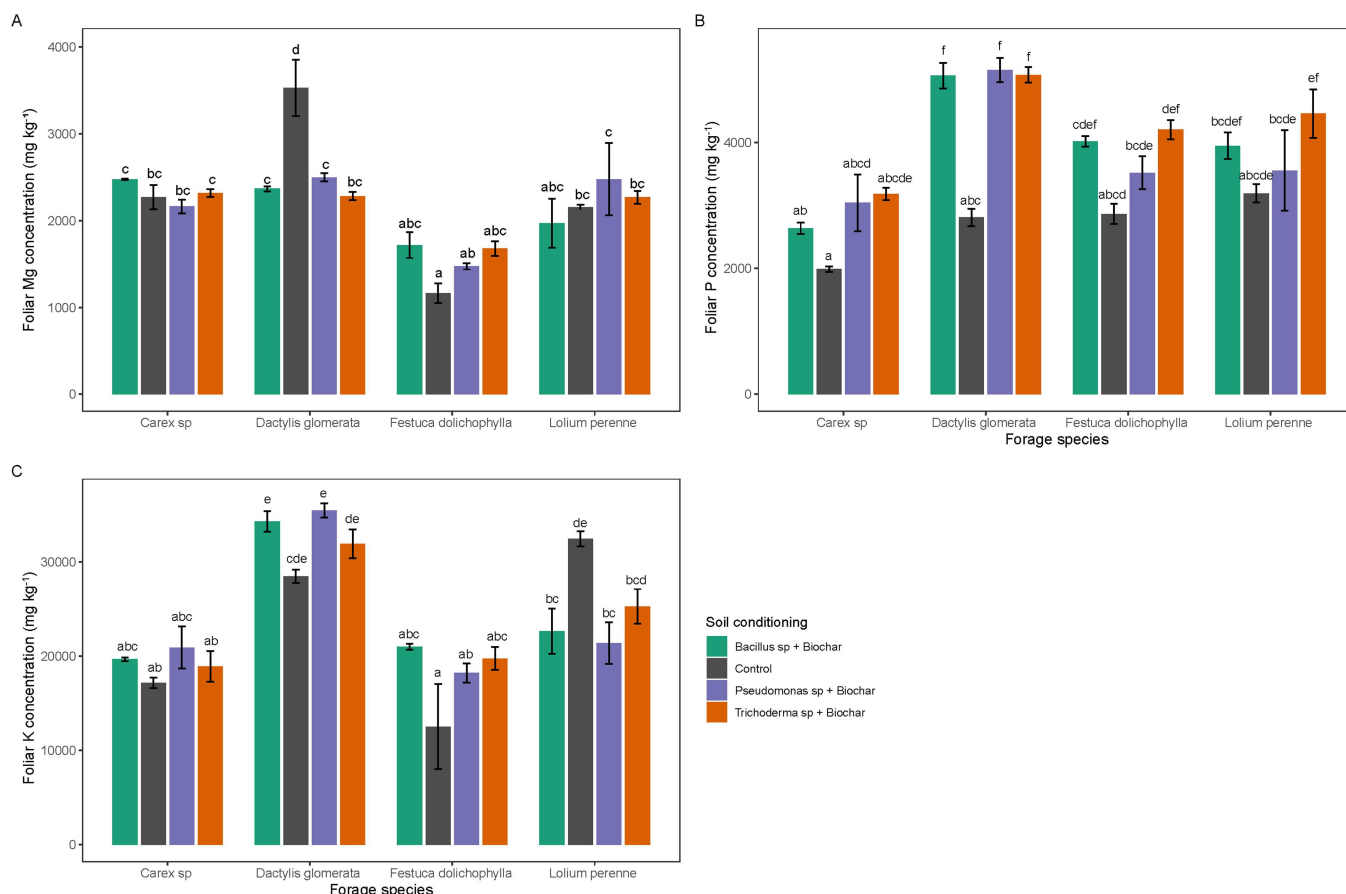


FIGURE 2 | Effects of soil conditioning on foliar macronutrient concentrations (Mg, P, and K) in high-Andean forage species. (A) Mg, (B) P, and (C) K. Bars represent means $\pm$ standard error. Different letters indicate significant differences among soil conditioning combinations according to Tukey's HSD test ($p < 0.05$).

TABLE 3 | Two-way ANOVA results for foliar concentrations of micronutrients (Fe, Mn, Cu, and Mo) as affected by forage species and soil conditioning.

Source of variation	df	Fe (F)	Fe (p)	Mn (F)	Mn (p)	Cu (F)	Cu (p)	Mo (F)	Mo (p)
Block	2	1.693	0.201	2.563	0.094	0.468	0.631	0.818	0.451
Forage species	3	3.578	0.025*	13.511	< 0.001***	5.612	0.004**	0.183	0.907
Soil conditioning	3	0.683	0.57	0.348	0.791	1.558	0.22	1.208	0.324
Forage species $\times$ Soil conditioning	9	0.944	0.503	0.292	0.972	1.799	0.11	1.5	0.193
Residual	30	—	—	—	—	—	—	—	—

Note: Values correspond to the F statistics (F) and associated probability values (p) obtained from a two-way ANOVA including block (random effect), forage species and soil conditioning (fixed factors), and their interaction. Significance levels are indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Dactylis glomerata and *Lolium perenne* recorded significantly higher concentrations compared with *Festuca dolichophylla* and *Carex sp.* For copper (Figure 3C), *Festuca dolichophylla* and *Dactylis glomerata* presented the highest values, while *Lolium perenne* showed the lowest concentrations.

3.4 | Forage Species-Dependent Responses of Foliar Trace Element Concentrations to the Application of Inoculated Biochar

The analysis of variance showed that forage species significantly influenced foliar concentrations of Ba, Sr, Co, and Na

($p < 0.01$), whereas Ni did not show significant differences among forage species (Table 4). The inoculated soil conditioning did not affect Ba, Ni, or Sr ($p > 0.05$), but exhibited highly significant effects on Co ($F = 54.82$; $p < 0.001$) and Na ($F = 26.40$; $p < 0.001$). Moreover, significant forage species $\times$ soil conditioning interactions were detected for Co ($p = 0.0326$) and Na ($p = 0.0134$), indicating a species-dependent response.

Consistent with the significant forage species effect, Figure 4 shows interspecific differences for Ba and Sr. For Ba (Figure 4A), *Festuca dolichophylla* exhibited the lowest concentrations, differing significantly from *Carex sp.* and *Lolium*

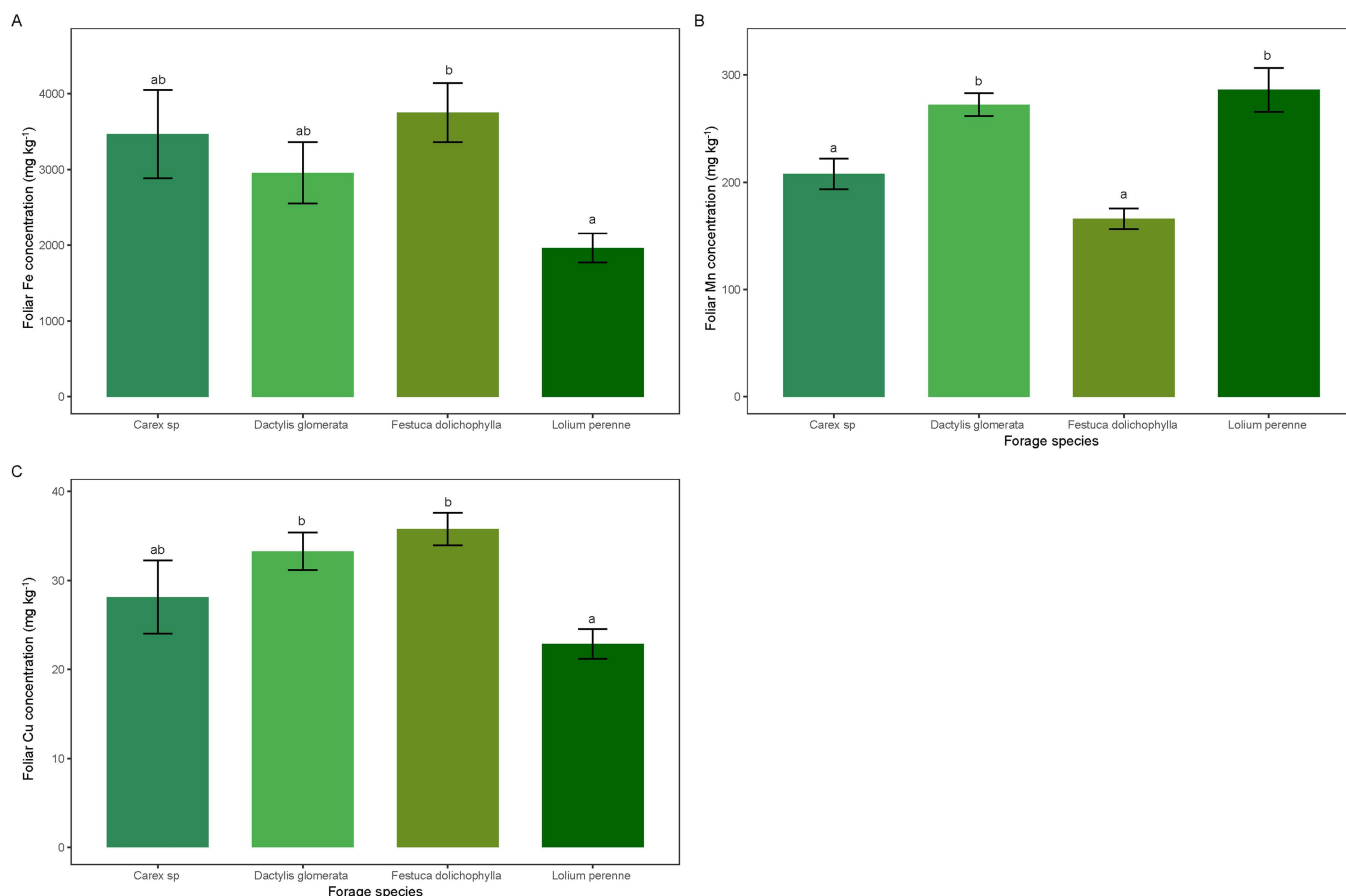


FIGURE 3 | Foliar micronutrient concentrations in four high-Andean forage species: (A) Fe, (B) Mn, and (C) Cu. Bars represent means $\pm$ standard error. Different letters indicate significant differences among forage species according to Tukey's HSD test ($p < 0.05$). No significant effects of soil conditioning or interaction were detected (Table 3).

TABLE 4 | Results of the two-way ANOVA results for foliar concentrations of trace elements (Ba, Ni, Sr, Co and Na) as affected by forage species and soil conditioning.

Source of variation	df	Ba (<i>F</i>)	Ba (<i>p</i>)	Ni (<i>F</i>)	Ni (<i>p</i>)	Sr (<i>F</i>)	Sr (<i>p</i>)	Co (<i>F</i>)	Co (<i>p</i>)	Na (<i>F</i>)	Na (<i>p</i>)
Block	2	0.443	0.646	1.283	0.292	1.566	0.226	0.696	0.506	0.38	0.687
Forage species	3	5.598	0.004**	1.193	0.329	10.421	<0.001***	20.367	<0.001***	32.522	<0.001***
Soil conditioning	3	1.856	0.158	0.93	0.438	0.713	0.552	54.822	<0.001***	26.396	<0.001***
Forage species $\times$ Soil conditioning	9	1.643	0.148	1.156	0.357	2.035	0.070	2.435	0.0326*	2.908	0.0134*
Residual	30	—	—	—	—	—	—	—	—	—	—

Note: Values correspond to the *F* statistics (*F*) and associated probability values (*p*) obtained from a two-way ANOVA including block (random effect), forage species and soil conditioning (fixed factors), and their interaction. Significance levels are indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

perenne, whereas *Dactylis glomerata* showed intermediate values. For Sr (Figure 4B), *Lolium perenne* recorded the highest concentrations and *Festuca dolichophylla* the lowest, with *Carex sp.* and *Dactylis glomerata* positioned in intermediate groups according to Tukey's test ($p < 0.05$).

The nature of the interactions detected for Co and Na is detailed in Figure 5. In the case of Co (Figure 5A), inoculated soil conditioning—particularly those involving *Pseudomonas* and *Trichoderma*—tended to increase foliar concentrations in several forage species compared with the control, although the

magnitude of the effect varied among forage species. For Na (Figure 5B), the control soil conditioning showed markedly higher concentrations in some forage species, whereas the application of inoculated biochar reduced foliar levels, revealing a consistent pattern of species-dependent modulation.

Overall, these results indicate that inoculated biochar did not broadly alter trace-element accumulation, but rather exerted selective effects on Co and Na, the magnitude of which was conditioned by the functional identity of the forage species.

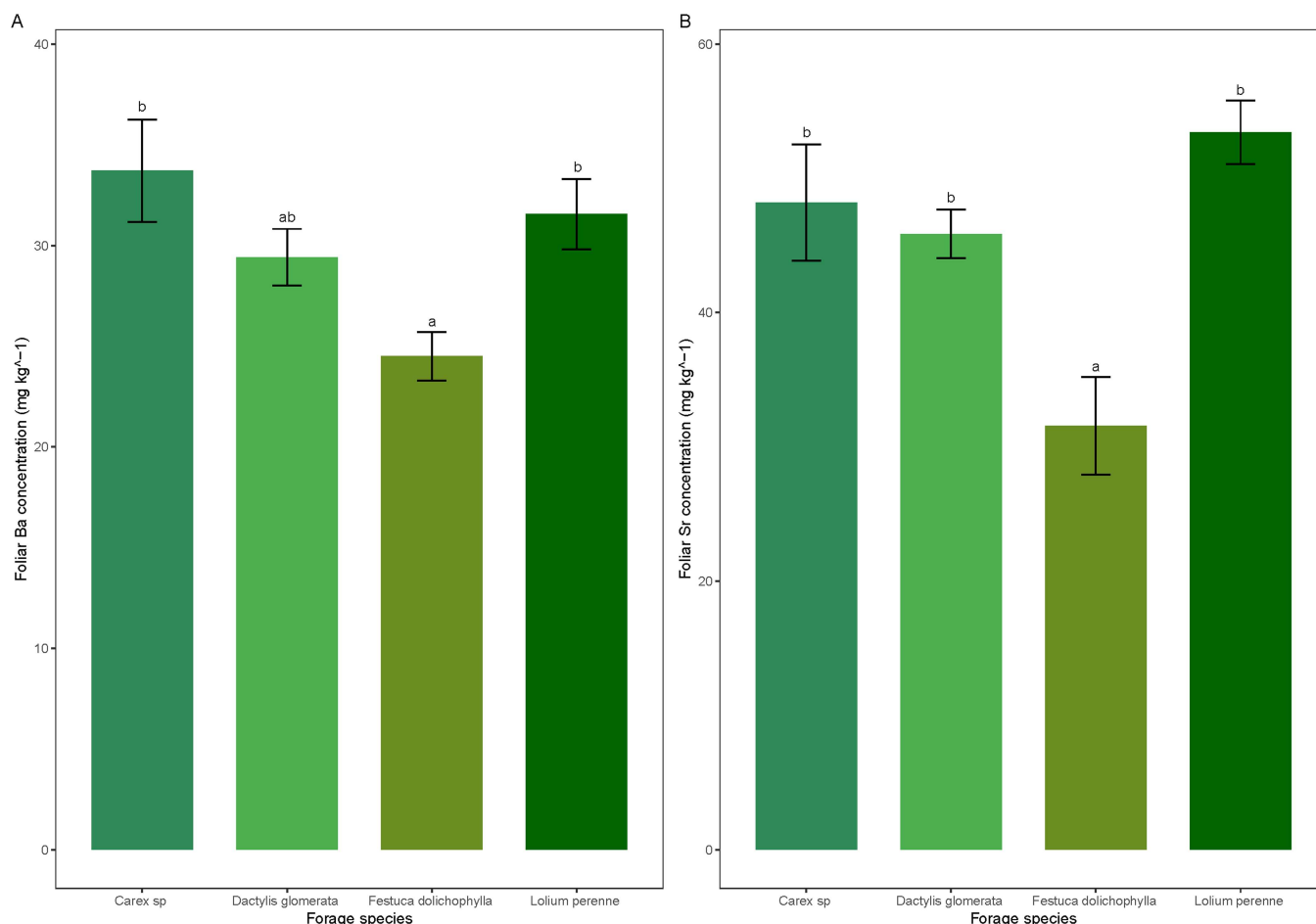


FIGURE 4 | Foliar concentrations of trace elements in four high-Andean forage species: (A) Ba and (B) Sr. Bars represent means $\pm$ standard error. Different letters indicate significant differences among species according to Tukey's HSD test ($p < 0.05$).

3.5 | Multivariate Reconfiguration of the Foliar Ionome in High-Andean Grasses Through Microorganism-Functionalized Biochar

The complexity of the interactions among biochar functionalization, microbial activity, and foliar nutritional response was synthesised through principal component analysis (PCA) (Figure 6) and a Z-score heatmap (Figure 7). This multivariate approach allowed the effects of soil conditioning to be distinguished from the intrinsic biological variability of the forage species, with the first two principal components explaining 50.3% of the total variance (PC1: 27.4% and PC2: 22.9%). This proportion of explained variance reflects the inherent complexity of ionomic datasets, suggesting that additional environmental, physiological, and soil-related factors not measured in this study may also contribute to the observed variability.

The PCA revealed two main gradients structuring the multivariate space of the ionomic data:

- **Taxonomic differentiation axis (PC1):** The horizontal axis separated the forage species according to their mineral acquisition patterns. A clear tendency toward segregation was observed, with introduced grasses (*Dactylis glomerata* and *Lolium perenne*) positioned toward the negative values

of PC1 and associated with the vectors of Ca, Mg, and Mn. This pattern may indicate a greater capacity for divalent cation accumulation and a tendency toward higher nutrient uptake in these forage species. In contrast, the native forage species *Festuca dolichophylla* was consistently located toward the positive extreme, suggesting a distinct mineral profile and a comparatively more conservative homeostatic response.

- **Soil conditioning response axis (PC2):** The vertical axis captured the influence of soil conditioning on the ionomic configuration. The vectors of Fe, Cu, and Ni were oriented toward the positive values of PC2, indicating their contribution to the variability associated with soil conditioning. In contrast, soil pH, phosphorus (P), and cobalt (Co) were aligned toward the negative values of PC2. This pattern suggests a potential association between acidity neutralisation and increased P availability, as well as the mobilisation of certain trace elements, although these relationships should be interpreted with caution, given the proportion of unexplained variance.

Likewise, the Z-score heatmap corroborated the vectorial trends observed in the PCA, enabling the identification of soil conditioning and species-specific mineral signatures:

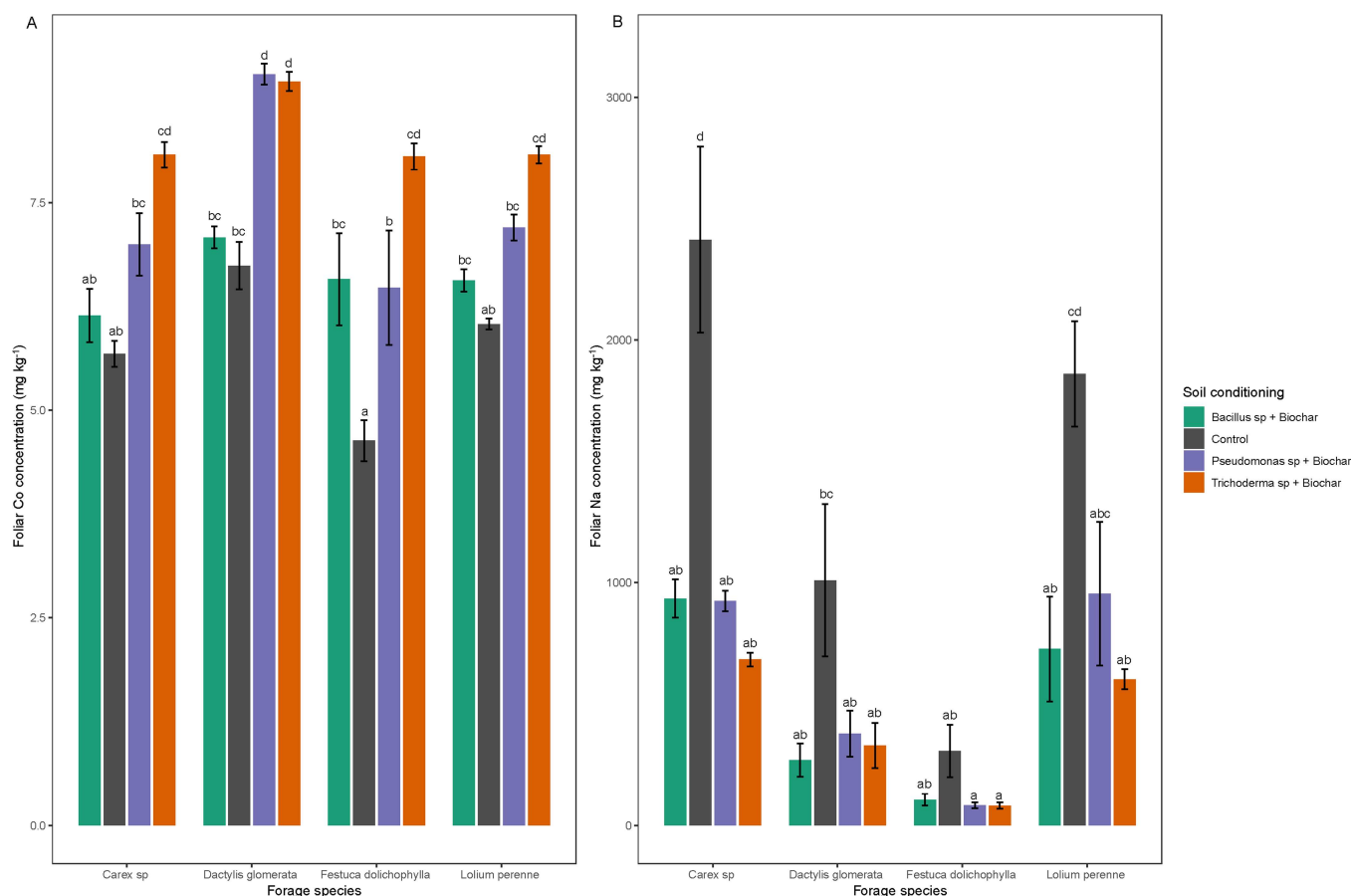


FIGURE 5 | Effects of soil conditioning on foliar trace-element concentrations (Co and Na) in high-Andean forage species. (A) Co and (B) Na. Bars represent means $\pm$ standard error. Different letters indicate significant differences among soil conditioning combinations within each forage species according to Tukey's HSD test ($p < 0.05$).

- **Microorganism-Induced Biofortification:** Inoculated soil conditioning, particularly *Trichoderma* sp. + biochar, exhibited Z-scores close to 1.0 for P and Co. This pattern confirms the effectiveness of microbial functionalization in reversing the critical P deficiency observed in the control group (Z-score < -1.0) and in enhancing the accumulation of functional elements above the overall experimental mean.
- **Micronutrient and Trace-Element Mobilisation:** The *Bacillus* sp. + biochar soil conditioning was distinguished by promoting the highest concentrations of molybdenum (Mo) and potassium (K). As the Mo vector points toward the upper left quadrant in the PCA, this soil conditioning appears to enhance the uptake of trace elements essential for enzymatic activity while maintaining lower barium (Ba) levels compared with the control.
- **Stability and Safety with Respect to Regulated Trace Elements:** Trace elements such as Ni, Fe, and Cu were positioned within a zone of moderate relative loading in the PCA, displaying predominantly neutral to low intensities in the biochar soil conditioning according to the heatmap. This stability indicates that the increase in soil pH did not compromise the uptake of essential metals nor induce excessive translocation of potentially phytotoxic elements. In contrast, control samples exhibited higher levels of Ba, Na,

and Fe, suggesting a less selective availability dynamic under baseline acidic conditions.

To explore the potential mechanism underlying these changes, linear regression analysis (Figure 8) was performed to evaluate the relationship between soil chemistry and the plant ionome. A weak positive association was observed between soil pH and foliar P concentration ($R = 0.26$); however, this relationship was not statistically significant, suggesting that the observed trend should be interpreted with caution and may not reflect a robust underlying process. Likewise, no significant correlations were detected between soil pH and micronutrients such as Fe and Mn ($p > 0.40$), indicating that the applied dose of 20 t ha^{-1} guinea pig manure biochar did not produce detectable imbalances in micronutrient uptake under the studied conditions.

In summary, the integration of both analytical approaches demonstrates that the application of guinea pig manure-derived biochar inoculated with microbial consortia acts as a modulator of the foliar ionome. This intervention reconfigures the mineral composition of high-Andean forages by optimising limiting macronutrients (P and K) and supplying functional elements such as cobalt (Co), while exerting a “filtering” effect that reduces the accumulation of non-essential trace elements such as sodium (Na) and barium (Ba).

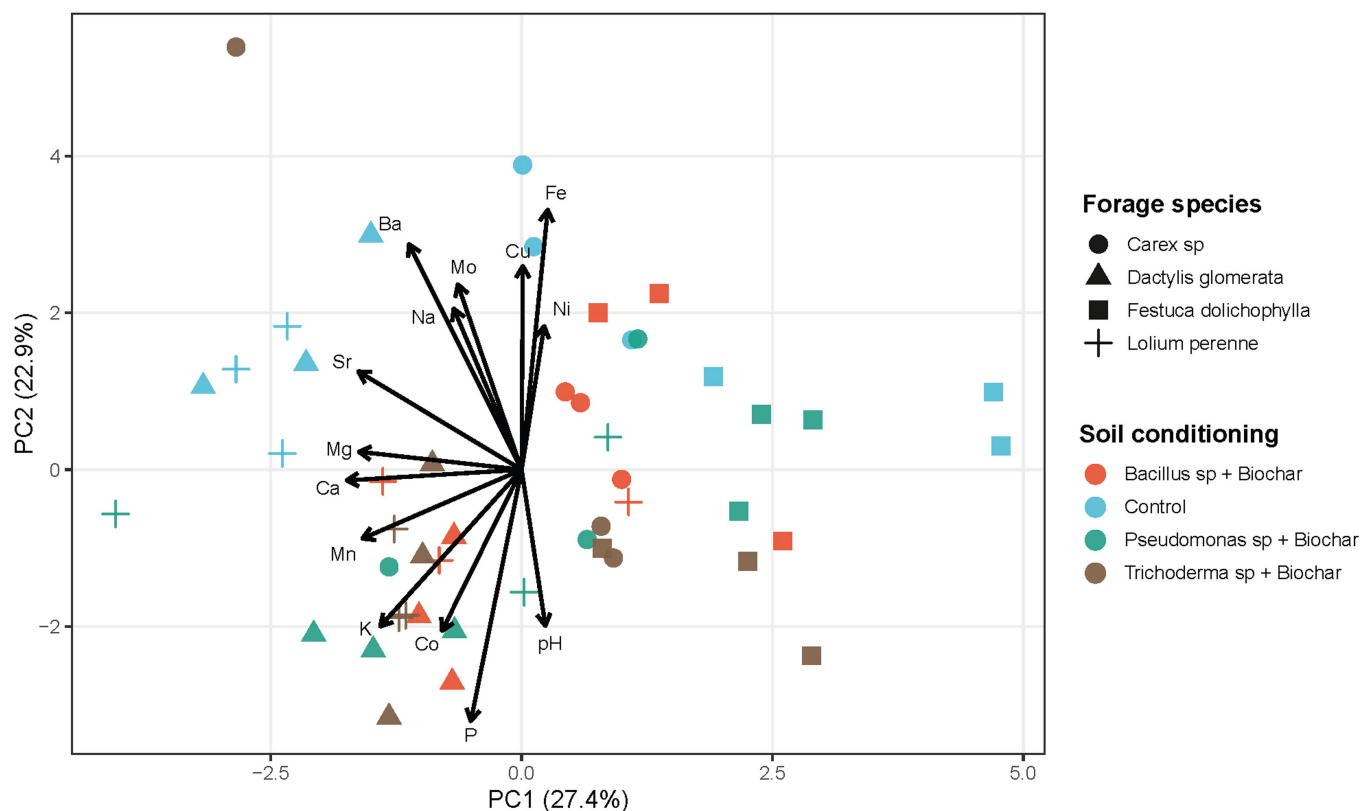


FIGURE 6 | Principal component analysis (PCA) of foliar ionic composition and soil pH showing the response of high-Andean forage species to soil conditioning.

4 | Discussion

4.1 | Inoculated Biochar as a Primary Modulator of Soil Acidity in High-Andean Soils

The results indicate that the inoculated biochar soil conditioning was the main factor driving soil pH modification, regardless of the forage species established. This effect is consistent with the widely documented behaviour of biochar as an alkalinising amendment, particularly in acidic soils of volcanic origin, where the presence of carbonates and basic oxides enhances soil buffering capacity (Liu et al. 2025; Geng et al. 2022). In high-Andean Andisols and Entisols, the increase in soil pH mediated by biochar reduces aluminium phytotoxicity and mitigates phosphorus fixation through Al-Fe complexes, thereby improving chemical soil fertility (Shetty et al. 2021).

The absence of a significant interaction between forage species and soil conditioning for soil pH suggests that the effect of biochar was predominantly edaphic rather than dependent on the species-specific rhizosphere. This finding is consistent with studies demonstrating that, at moderate to high application rates, biochar exerts a dominant geochemical influence on the soil-solution system, overriding variations associated with differences in root exudation patterns or species-specific microbial activity (von Haden et al. 2024).

Comparable responses have been reported in volcanic and highly weathered soils, where biochar application resulted in significant increases in soil pH and reductions in exchangeable acidity, particularly in systems dominated by Al-Fe complexes (Bolan et al. 2023; Barrezueta-Unda, Bonilla, and Hidalgo 2023).

However, the magnitude of the increase observed in the present study was moderate compared with trials using higher application rates ($> 30\text{--}60\text{ t ha}^{-1}$), in which more pronounced pH increases were documented, but also a greater risk of cationic imbalances and alterations in micronutrient availability (Khan et al. 2021; Xu et al. 2022). In this context, the response recorded suggests that the applied dose functioned as an efficient corrective amendment without inducing abrupt chemical transformations, which is particularly relevant in high-Andean ecosystems characterised by structural fragility and limited edaphic resilience. Similar responses have recently been reported in degraded soils amended with biochar, where intermediate application rates promoted improvements in soil functioning and microbial ecological organisation without causing major disruptions to ecosystem stability (dos Santos et al. 2026). Together, these findings support the use of biochar as a selective soil-conditioning strategy capable of enhancing soil quality while maintaining overall ecosystem functionality.

4.2 | Macronutrient Availability and Responses Dependent on Functional Identity

The increase in foliar phosphorus observed under inoculated soil conditioning can be attributed to the reduction of exchangeable Al and the consequent mitigation of P fixation in Al-Fe complexes (Bornø et al. 2018). In addition, microbial functionalization of biochar enhanced phosphorus mineralisation through increased phosphatase activity and the production of organic acids (Zhang et al. 2025), thereby improving soil fertility in these volcanic-derived soils.

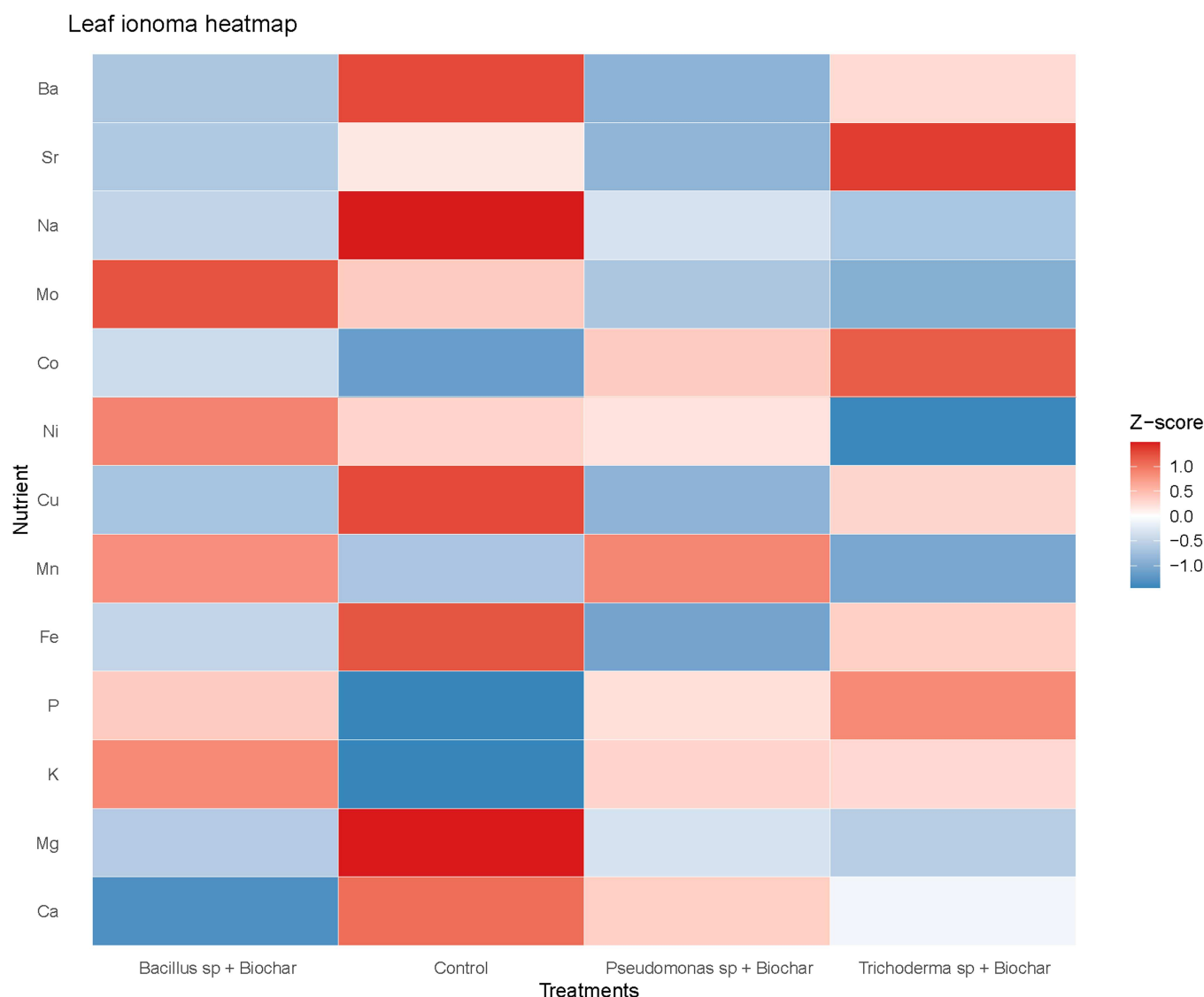


FIGURE 7 | Heatmap of the foliar ionome showing relative changes in the concentrations of macro-, micro-, and trace elements in response to microorganism-inoculated biochars.

However, the magnitude of the response was species-dependent, particularly for Mg and K, where significant forage species $\times$ soil conditioning interactions were detected. This pattern indicates that improvements in soil conditions are not uniformly translated into foliar nutrient accumulation, but are instead modulated by physiological strategies governing nutrient acquisition and mineral homeostasis. Interspecific variation in nutrient uptake and translocation has been widely documented and is associated with differences in root architecture, transporter density, and vacuolar storage capacity (Tränkner, Tavakol, and Jákli 2018; Xie et al. 2024). In this context, introduced forage species may exhibit more acquisitive strategies under nutrient-enriched conditions, whereas native forage species such as *Festuca dolichophylla* may display a more conservative regulation of their ionome.

This differential pattern is consistent with studies demonstrating that foliar responses to increased nutrient availability depend strongly on species functional identity and nutrient acquisition strategy (Moenclaey et al. 2022). Improvements in

soil fertility do not necessarily translate into proportional increases in tissue nutrient concentrations because homeostatic regulation mechanisms and transporter-mediated adjustments regulate nutrient uptake (Yao et al. 2020). Accordingly, the interspecific variability observed in the present study can be interpreted within the framework of plant nutrient economics, whereby acquisitive forage species respond more strongly to enhanced fertility, whereas conservative species prioritise ionic stability and nutrient-use efficiency (Ma et al. 2024).

4.3 | Micronutrient Stability Under Conditions of Moderate Alkalinization

A relevant finding was the absence of significant soil conditioning effects on foliar Fe, Mn, and Cu concentrations, as well as the lack of strong negative correlations between soil pH and these micronutrients. In acidic soils, abrupt increases in pH can induce Fe and Mn deficiencies due to reduced solubility, leading to their transformation into insoluble hydroxides or

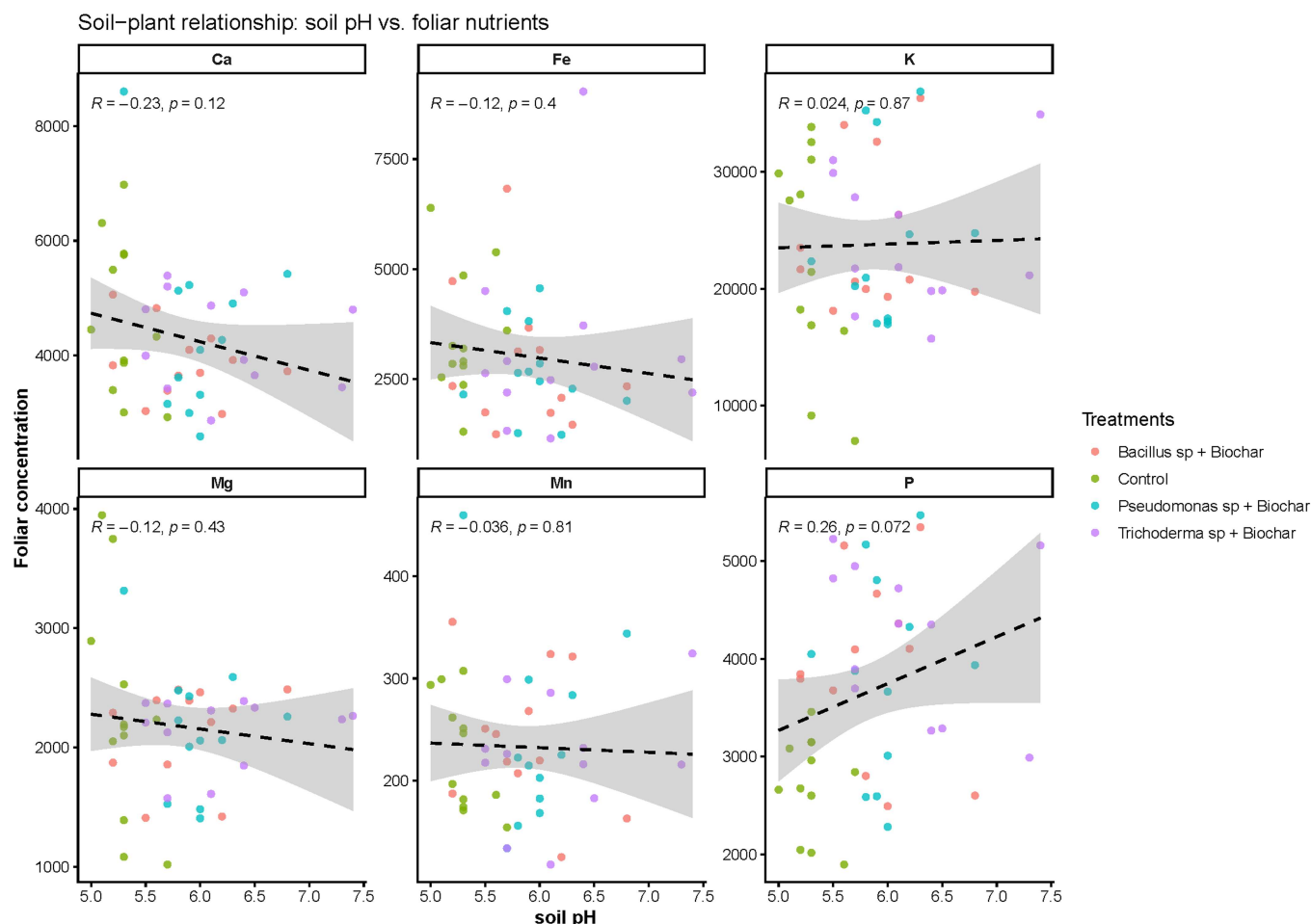


FIGURE 8 | Correlations between soil pH and foliar concentrations of macro- and micronutrients in forage species exposed to microorganism-inoculated biochars.

oxides (Haas, Boschi, and Grannas 2019). However, the pH shift observed in the present study (~5.2 to ~6.0) remained within a range in which the availability of essential micronutrients is not critically constrained (Ferrarezi et al. 2022).

These results suggest that the applied dose (20 t ha^{-1}) functioned as a corrective amendment without inducing nutritional imbalances, supporting the concept that biochar can enhance the fertility of acidic soils without generating adverse effects on the uptake of essential metals (Gale and Thomas 2019). The stability of foliar Fe, Mn, and Cu further indicates that the system reconfiguration was selective rather than reflecting a generalised alteration of the plant ionome.

Some studies have reported reductions in Fe and Mn availability following abrupt pH increases induced by biochar, particularly when the shift exceeds the slightly acidic range (Ma et al. 2025; Wu et al. 2018). However, research conducted in acidic soils experiencing moderate pH increases has shown that micronutrient concentrations can remain within physiologically adequate ranges when soil correction does not surpass the critical solubility threshold (Ohyama et al. 2023). The stability observed in the present study is consistent with these findings and suggests that the system maintained redox conditions and nutrient speciation compatible with sustained uptake

of essential metals, thereby avoiding secondary effects associated with over-alkalinization.

4.4 | Selective Modulation of Trace Elements: Co and Na as Functional Indicators

The differential mobilisation of cobalt by *Trichoderma* sp. is particularly noteworthy. This fungus can enhance Co availability through rhizosphere acidification and the production of siderophores, which, although primarily involved in Fe acquisition, may also chelate and mobilise other transition metals such as Co (Yang et al. 2019). Recent evidence further highlights that *Trichoderma* acts as a multifunctional rhizosphere regulator, influencing nutrient competition, microbial community dynamics, and metal acquisition processes through a combination of direct and indirect mechanisms (dos Santos 2025). In high-Andean pastoral systems, where livestock depend almost exclusively on forage resources, this increase in foliar Co concentrations could translate into improved vitamin B12 synthesis by the ruminal microbiota (Siregar, Salas-Solis, and Faciola 2025).

Conversely, the sodium exclusion observed under *Trichoderma* sp. suggests a mechanism involving cationic competition or the

upregulation of high-affinity K^+ transporters that discriminate against Na^+ , a response similar to that reported in systems where biochar mitigates saline stress (Akhtar, Andersen, and Liu 2015). This selective response, dependent on microbial identity, reinforces the notion that guinea pig manure-derived biochar acts not as a generalised immobilisation agent (Kujawska, Wojtaś, and Charmas 2024), but rather as a modulator of ionic balance (Adhikari, Moon, and Timms 2024; De la Rosa et al. 2022) the direction and magnitude of which are governed by the inoculated microorganism.

4.5 | Multivariate Reconfiguration of the Ionome: Evidence of a Stable yet Modulable Architecture

Multivariate analysis (PCA and heatmap) enabled the integration of individual responses within a systemic framework. The separation of axes associated with metallic micronutrients and macronutrients confirms that the plant ionome operates as a highly interconnected network, in which elements do not vary independently (Fikas et al. 2019; Chaparro et al. 2018).

Beyond this general pattern, the PCA revealed biologically meaningful gradients associated with species-specific nutrient acquisition strategies. The positioning of *Dactylis glomerata* and *Lolium perenne* along the axis associated with Ca, Mg, and Mn suggests a more acquisitive strategy, characterised by a greater capacity for divalent cation uptake. In contrast, *Festuca dolichophylla* was located at the opposite end of this gradient, indicating a more conservative nutrient-use strategy, potentially associated with tighter ion homeostasis and adaptation to nutrient-limited acidic soils. These contrasting patterns highlight that forage species differ not only in their elemental composition but also in their physiological regulation of nutrient uptake and internal balance.

The vertical gradient (PC2) further reflects the influence of soil conditioning on ionic configuration. The association between higher soil pH and phosphorus availability supports the hypothesis that partial neutralisation of soil acidity was a key driver of improved macronutrient acquisition. At the same time, the redistribution of trace elements such as Fe, Cu, and Ni suggests that biochar and microbial inoculation may have altered element mobility and plant uptake pathways. These patterns are consistent with shifts in ion regulation mechanisms, including selective uptake, internal redistribution, and exclusion processes that enable plants to maintain physiological stability under changing edaphic conditions.

However, the absence of complete segregation in the multivariate space indicates that the observed changes occurred within the boundaries of elemental homeostasis rather than representing a complete restructuring of the ionome. This supports the view that biochar-based interventions modulate, rather than override, intrinsic nutrient regulation mechanisms. Consistent with previous ionomics studies, the results suggest that edaphic perturbations induce coordinated adjustments in subsets of elements while maintaining overall ionic stability (He et al. 2023; Zhang et al. 2021). Together with the moderate positive association between soil pH and phosphorus, these

findings reinforce that soil–plant interactions are governed by complex geochemical, microbial, and physiological controls (Xia et al. 2024; Wang et al. 2024). Consequently, the multivariate patterns observed here reflect a stable yet modulable ionic architecture through which high-Andean forage species adjust nutrient acquisition and ion regulation in response to soil acidity and resource availability.

Taken together, the results indicate that inoculated biochar promoted a selective ionic reconfiguration characterised by increased phosphorus availability, stable concentrations of essential micronutrients, and targeted modulation of trace elements such as Co and Na. The association of PC2 with soil pH, phosphorus, and cobalt suggests that soil conditioning influenced nutrient availability and trace-element dynamics, whereas species-specific nutrient acquisition strategies largely explained variation along PC1. These findings indicate that microorganism-functionalized biochar acts as a selective modulator of the foliar ionome, enhancing nutrient acquisition while maintaining elemental homeostasis and nutritional balance in high-Andean forage systems.

4.6 | Mechanistic Basis of Nutrient Mobilisation and Ion Homeostasis Under Microorganism-Functionalized Biochar

The observed increases in foliar phosphorus (P) and cobalt (Co), together with the reduction in sodium (Na), indicate that microorganism-functionalized biochar operates through complementary geochemical and biological mechanisms regulating nutrient availability and plant ion homeostasis (Figure 9).

The increase in P is likely associated with the combined effects of biochar-induced pH correction and microbial activity. Partial neutralisation of soil acidity may have reduced P fixation by Al–Fe complexes, while inoculated microorganisms could further enhance P mobilisation through phosphatase activity, organic acid production, and the mineralisation of organic P pools (Boat Bedine et al. 2022; Qin et al. 2025; Fan et al. 2025). Likewise, the increase in foliar Co concentrations may reflect microbial-mediated mobilisation of trace elements through siderophore production and other chelating mechanisms that enhance the solubility and uptake of transition metals. These processes are consistent with the multifunctional role of *Trichoderma* in rhizosphere regulation, nutrient competition, microbial community modulation, and metal acquisition under environmentally constrained conditions (dos Santos 2025; Pradhan et al. 2025; Bi et al. 2010; Ahmed and Holmström 2014). In contrast, the reduction in foliar Na suggests improved ion selectivity under enhanced soil fertility conditions. Increased acquisition of essential nutrients, particularly K^+ , may have favoured mechanisms that restrict Na accumulation and maintain ionic balance through selective transport and compartmentalisation processes (Li et al. 2024; Feng et al. 2018).

Overall, these findings support the interpretation that the biochar–microbe system functions through coordinated interactions between soil chemical modification and rhizosphere biological activity, promoting a selective reconfiguration of the plant ionome while preserving elemental homeostasis. This integrative mechanism

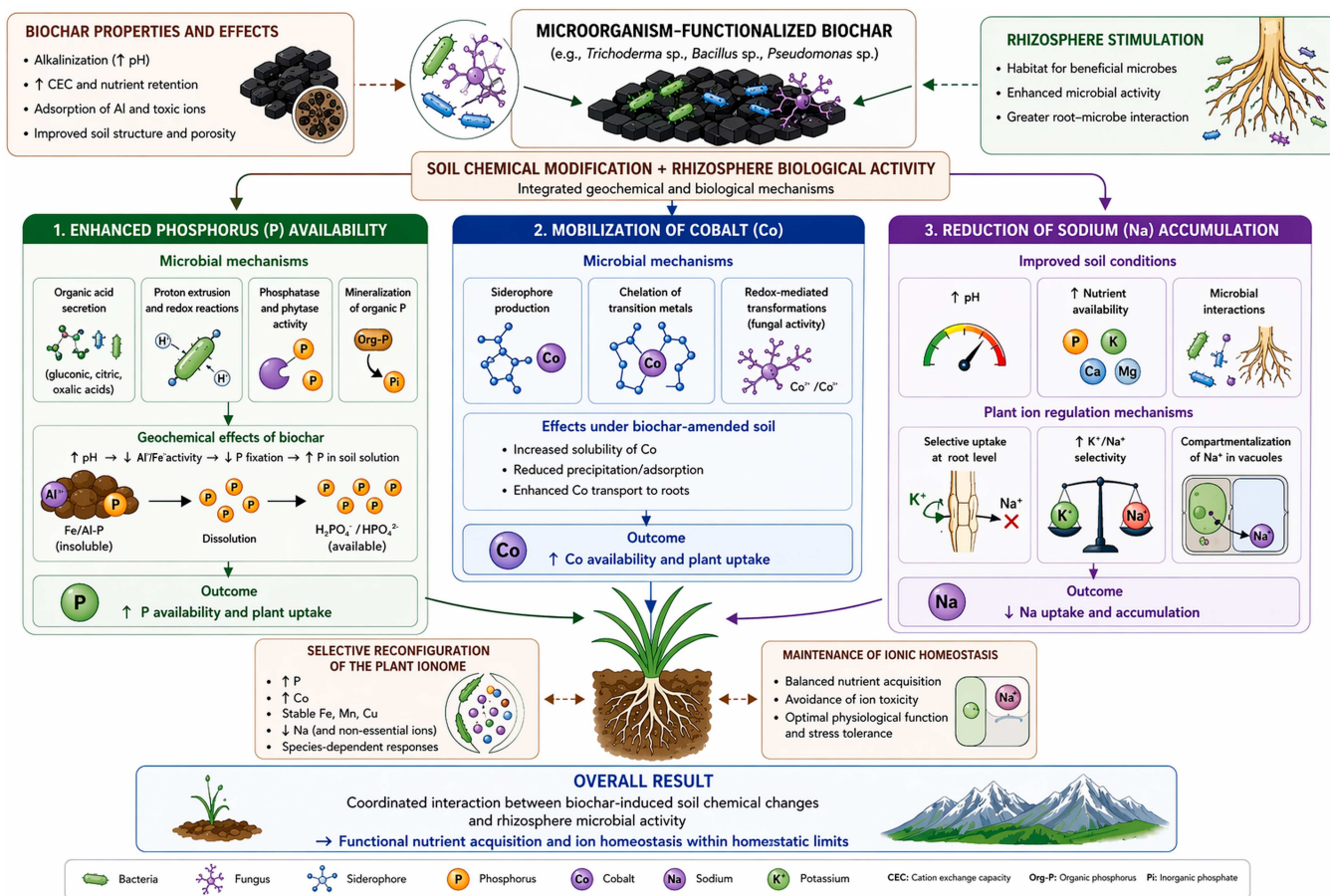


FIGURE 9 | Mechanistic framework of ionic reconfiguration in high-Andean forage species induced by microorganism-functionalized biochar.

highlights the potential of microorganism-functionalized biochar to enhance nutrient acquisition and nutrient-use efficiency in high-Andean agroecosystems.

4.7 | Limitations and Future Research Perspectives

Despite the robustness of the experimental design, several methodological limitations should be considered when interpreting the results. First, the relatively small plot size (4 m²) may allow residual edge effects under field conditions, despite the use of buffer zones. Second, the biochar application rate (20 t ha⁻¹), although effective for evaluating soil conditioning responses, may limit direct extrapolation to large-scale agricultural systems because of economic and logistical constraints. Finally, measurements were conducted at a single time point, restricting the assessment of temporal dynamics in soil properties and plant ionic responses.

A key limitation of this study is the lack of direct assessment of microbial establishment following inoculation. The survival, persistence, and root colonisation of the introduced microorganisms under high-Andean field conditions were not evaluated, limiting mechanistic interpretation of their contribution to the observed responses. Although changes in soil pH and foliar ionic composition suggest a functional role of

microbial activity, these effects cannot be unequivocally attributed to the successful establishment of the inoculated strains. This limitation is particularly relevant in high-altitude environments, where low temperatures, intense solar radiation, and edaphic constraints may affect microbial viability and activity.

Despite these limitations, the consistent responses observed across soil-conditioning treatments suggest that inoculated biochar influenced nutrient mobilisation and ionic regulation. Future studies should incorporate direct validation approaches, including microbial population monitoring through quantitative PCR (qPCR), high-throughput sequencing (16S rRNA and ITS), and strain-specific molecular markers to assess persistence and activity. Multi-temporal sampling and evaluation of different biochar application rates under field-scale conditions would further improve understanding of system dynamics and scalability. Such approaches would provide a stronger mechanistic framework for elucidating biochar-microbe-plant interactions in high-Andean ecosystems.

5 | Conclusion

This study provides mechanistic evidence that microorganism-functionalized biochar acts as a regulator of plant ionic composition in high-Andean grasslands, moving beyond its

conventional role as a soil amendment. By demonstrating that microbial identity—particularly *Trichoderma* sp.—drives selective nutrient acquisition and ionic balance, this work advances current understanding of plant–soil–microbe interactions and highlights the importance of microbe-specific functional traits in shaping plant nutrition.

From an applied perspective, these findings introduce a novel framework for microbiome-assisted nutrient management in acidic and high-altitude agroecosystems. The ability to enhance beneficial elements such as phosphorus while limiting potentially harmful ions like sodium represents a promising strategy to improve forage quality, nutrient use efficiency, and system resilience under edaphic and climatic constraints.

More broadly, this study opens new research avenues focused on the functional optimisation of biochar–microbe combinations, the persistence and activity of inoculated microorganisms under extreme conditions, and the integration of ionic approaches into sustainable soil management. These insights contribute to the development of targeted, climate-resilient agricultural practices aimed at restoring productivity in degraded soils while maintaining ecological balance.

Author Contributions

Alberto Arias-Arredondo: study conception, experimental design, data collection, analysis of results, and manuscript drafting. **Melina Lopez Rodríguez:** assistance with data collection and methodology. **Juancarlos Cruz Luis:** resource allocation, supervision, and manuscript drafting. **Richard Solórzano-Acosta:** critical review of the manuscript, evaluation of the consistency of the results and discussion, and assistance with the final interpretation of the results. All authors read, reviewed, and approved the final manuscript.

Acknowledgements

The authors sincerely thank the Vicco Peasant Community for granting access to the study area and for their valuable support during the fieldwork. This research was funded by the CUI 2487112 INIA project, Research and technology transfer services improvement in the management and recovery of degraded agricultural soils and irrigation water for small and medium-scale agriculture in the departments of Lima, Ancash, San Martín, Cajamarca, Lambayeque, Junín, Ayacucho, Arequipa, Puno and Ucayali. This research was funded by the CUI 2487112 INIA project, Research and technology transfer services improvement in the management and recovery of degraded agricultural soils and irrigation water for small and medium-scale agriculture in the departments of Lima, Ancash, San Martín, Cajamarca, Lambayeque, Junín, Ayacucho, Arequipa, Puno and Ucayali.

Use of Artificial Intelligence Statement

Artificial intelligence-based tools were used only in the preparation of Figure 9. The figure was generated with the assistance of AI-based tools and subsequently reviewed, edited, and validated by the authors. No artificial intelligence tools were used for the writing, analysis, interpretation of results, or any other part of the manuscript. The authors take full responsibility for the content and integrity of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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