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Microbial respiration and soil acidity under liming and organomineral amendment

Respiración microbiana y acidez del suelo bajo encalado y enmienda orgánico mineral

Respiração microbiana e acidez do solo sob calagem e enmienda organomineral

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Abstract

Soil acidity severely limits biological activity in high Andean systems, where aluminum toxicity and low pH reduce agricultural productivity and constrain microbial processes. In this context, the effect of calcium carbonate ($CaCO_3$), applied both individually and in combination with a commercial organo-mineral amendment, was evaluated on soil pH, exchangeable aluminum, and microbial respiration in an acidic soil collected from an agricultural farm located in the mountainous region of Pamplona (Norte de Santander), through a 15 day controlled incubation conducted between March and May 2021. A completely randomized design with four treatments (control, 100 % $CaCO_3$, 50 % $CaCO_3$, and 50 % $CaCO_3$ + amendment) and five replicates per treatment was used, measuring chemical variables and respiration at multiple incubation times; statistical analyses included ANOVA, Spearman correlations, and multiple linear regressions. Results showed that $CaCO_3$ significantly increased pH (up to +0.62 units at the full dose) and reduced exchangeable aluminum only in this treatment, while all amended treatments enhanced microbial respiration, particularly during early stages, with a tendency toward a stronger response in the organic combination. However, multivariate analysis revealed that chemical variables did not independently explain respiratory variability, highlighting the predominance of the integrated treatment effect. It is concluded that liming, especially when combined with organic amendments, corrects chemical acidity and revitalizes microbial activity through systemic effects, with practical implications for the sustainable management of high Andean soils.

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Resumen

La acidez del suelo limita de manera significativa la actividad biológica en sistemas alto andinos, donde la toxicidad por aluminio y el bajo pH reducen el rendimiento agrícola y restringen los procesos microbianos. En este contexto, se evaluó el efecto del carbonato de calcio ($CaCO_3$), aplicado de forma individual y en combinación con una enmienda orgánico mineral comercial, sobre el pH, el aluminio intercambiable y la respiración microbiana en un suelo ácido procedente de una finca agrícola ubicada en la zona montañosa de Pamplona (Norte de Santander), mediante una incubación controlada de 15 días realizada entre marzo y mayo de 2021. Se utilizó un diseño completamente aleatorizado con cuatro tratamientos (control, 100 % $CaCO_3$, 50 % $CaCO_3$ y 50 % $CaCO_3$ + enmienda), cada uno con cinco repeticiones, midiendo variables químicas y respiración en distintos momentos; el análisis estadístico incluyó ANOVA, correlaciones de Spearman y regresiones lineales múltiples. Los resultados mostraron que el $CaCO_3$ incrementó significativamente el pH (hasta +0,62 unidades en la dosis completa) y redujo el aluminio intercambiable únicamente en ese tratamiento, mientras que todos los tratamientos con enmiendas aumentaron la respiración microbiana, especialmente en las fases iniciales, con una tendencia a mayor respuesta en la combinación orgánica. Sin embargo, el análisis multivariado evidenció que las variables químicas no explicaron de forma independiente la variabilidad respiratoria, lo que sugiere el predominio del efecto integral del tratamiento. Se concluye que el encalado, particularmente cuando se combina con enmiendas orgánico mineral, corrige la acidez química y estimula la actividad microbiana mediante efectos sistémicos, con implicaciones prácticas para el manejo sostenible de suelos alto andinos.

Palabras clave: incubación controlada, actividad biológica, reacción del suelo, suelos alto andinos.

Resumo

A acidez do solo limita severamente a atividade biológica em sistemas alto-andinos, onde a toxicidade por alumínio e o baixo pH reduzem a produtividade agrícola e restringem os processos microbianos. Nesse contexto, avaliou-se o efeito do carbonato de cálcio ($CaCO_3$), aplicado de forma isolada e em combinação com uma emenda organo-mineral comercial, sobre o pH, o alumínio trocável e a respiração microbiana em um solo ácido proveniente de uma propriedade agrícola localizada na região montanhosa de Pamplona (Norte de Santander), por meio de uma incubação controlada de 15 dias, realizada entre março e maio de 2021. Utilizou-se um delineamento inteiramente casualizado com quatro tratamentos (controle, 100 % $CaCO_3$, 50 % $CaCO_3$ e 50 % $CaCO_3$ + emenda), com cinco repetições por tratamento, medindo variáveis químicas e respiração em diferentes momentos de incubação; as análises estatísticas incluíram ANOVA, correlações de Spearman e regressões lineares múltiplas. Os resultados mostraram que o $CaCO_3$ elevou significativamente o pH (até +0,62 unidades na dose completa) e reduziu o alumínio trocável apenas nesse tratamento, enquanto todos os tratamentos com emendas aumentaram a respiração microbiana, especialmente nas fases iniciais, com tendência de maior resposta na combinação orgânica. No entanto, a análise multivariada indicou que as variáveis químicas não explicaram de forma independente a variabilidade respiratória, evidenciando o predomínio do efeito integrado do tratamento. Conclui-se que a calagem, especialmente quando combinada com emendas orgânicas, corrige a acidez química e revitaliza a atividade microbiana por meio de efeitos sistêmicos, com implicações práticas para o manejo sustentável de solos alto-andinos.

Palavras-chave: incubação controlada, atividade biológica, reação do solo, solos alto-andinos.

Introduction

Soil acidity represents one of the major constraints on fertility and biogeochemical functioning in tropical mountain agroecosystems. In these environments, pH values below 5.5 promote the solubilization of aluminum into toxic trivalent forms, which inhibit root growth, restrict the uptake of essential nutrients, and disrupt microbial carbon and nitrogen cycling (Ofoe *et al*., 2023).

In the Colombian Andean region, intense leaching driven by high precipitation accelerates base cation losses and progressive acidification, thereby increasing exchangeable aluminum saturation in medium- to fine-textured soils, such as those predominant in Pamplona (Norte de Santander). These conditions ultimately compromise both agricultural productivity and soil biological vitality (Cruz *et al*., 2022; Flórez and Ochoa, 2022; IGAC, 2015).

Under this scenario, liming with calcium carbonate is widely recognized as a conventional corrective practice, capable of neutralizing soil acidity through proton consumption and the precipitation and/or complexation of toxic aluminum via well-established chemical reactions (Bolan *et al*., 2003). However, its effectiveness depends not only on application rate and reaction time, but also on interactions with soil organic matter and the inherent microbial community. Although numerous studies have evaluated the chemical effects of liming, biological indicators such as basal microbial respiration have received comparatively limited attention, particularly in high Andean environments, where moderate levels of organic matter may modulate soil responses in complex and not yet fully understood ways (Malik *et al*., 2018).

Microbial respiration provides an integrative window into heterotrophic activity and the mineralization of soil organic carbon, regulated by factors such as pH, the availability of labile substrates, and the taxonomic composition of the microbial community (Malik *et al*., 2018). Organic amendments, by supplying readily degradable carbon and additional buffering capacity, may enhance these effects, generating synergies with $CaCO_3$ that warrant systematic investigation. However, a critical knowledge gap persists: few studies integrate chemical dynamics (pH, Al^{3+}) and biological responses under controlled incubation in representative Andean soils, leaving unresolved how these combined management practices affect soil functional dynamics in the short term.

In this context, the present study evaluated the effect of liming alone and in combination with a commercial organo-mineral amendment on soil pH, exchangeable aluminum, and microbial respiration in a typical acidic soil from Pamplona, using a 15 day incubation period designed to capture the initial reaction phase. This approach aims to provide locally relevant evidence to optimize remediation practices in high Andean systems, where chemical adjustments have direct implications for soil microbial activity and overall edaphic functioning.

Materials and methods

Study area and soil sampling

The experiment was conducted using soil collected from an agricultural field in the municipality of Pamplona (Norte de Santander, Colombia), located at 2,200 m above sea level. The site is characterized by a mean annual precipitation of 1,863 mm and an average temperature of 11.3 °C, conditions typical of high Andean environments prone to base cation leaching and soil acidification (Villamizar *et al*., 2024; IGAC, 2015). Soil samples were collected prior to planting and the routine application of agricultural lime, with the aim of capturing the baseline chemical status of the soil under conventional management. Samples were taken from a depth of 0–20 cm, comprising five composite samples formed from 10 individual subsamples systematically collected on a 10 × 10 m grid within the plot (total area of 100 m^2). Subsamples were homogenized in the field, sieved to < 2 mm in the laboratory, and stored at 4 °C until analysis (maximum 48 h).

Experimental design and treatments

The experiment was established under a completely randomized design with four treatments and five replicates: T0 (unamended control), T1 (100 % of the $CaCO_3$ dose), T2 (50 % of the $CaCO_3$ dose), and T3 (50 % of the $CaCO_3$ dose combined at a 1:1 ratio with a commercial organo-mineral amendment).

The $CaCO_3$ dose was calculated based on initial exchangeable aluminum, expressed as calcium carbonate equivalent (CCE = 100), following McLean (1982). Conversion to $kg.ha^{-1}$ accounted for bulk density, sampling depth (0–20 cm), and soil mass per hectare. Final application rates corresponded approximately to 1.5 $t.ha^{-1}$ for T1 and 0.75 $t.ha^{-1}$ for T2 and T3, equivalent to 0.15 g and 0.075 g per 200 g of soil, respectively.

The organo-mineral amendment consisted primarily of composted poultry manure, polyhalite, phosphate rock, and sulfur sources, with an organic carbon content of 10.9 %, CaO of 21.4 %, and a near-neutral pH (6.98).

Each experimental unit consisted of 200 g of homogenized soil adjusted to field capacity and incubated for 15 days at 25 °C in airtight containers. This period was selected to capture the early reaction phase of liming, during which significant changes in pH, exchangeable aluminum, and respiratory activity occur under controlled conditions (Giang *et al*., 2024; Massaccesi *et al*., 2024).

Soil physical and chemical analyses

Soil texture was determined using the hydrometer method; gravimetric moisture content was measured by oven-drying at 105 °C for 24 h; and bulk density was determined using the core (cylinder) method (Gee and Bauder, 1986; Gardner, 1986; Blake and Hartge, 1986).

Soil pH and electrical conductivity (EC) were measured in a 1:2 (w/v) soil-to-water suspension using a pH meter and a conductivity meter, respectively, with EC expressed in $\mu S.cm^{-1}$. Exchangeable aluminum was determined by extraction with 1 $mol.L^{-1}$ KCl followed by titration (McLean, 1982).

Soil organic matter was estimated by loss-on-ignition at 450 °C, and organic carbon was calculated using the factor 1.724 (Westman *et al*., 2006; Pribyl, 2010). Chemical variables (pH, EC, and exchangeable Al) were evaluated before and after incubation.

Determination of soil microbial respiration

Basal microbial respiration was determined during incubation using the alkali trap method for CO_2 capture described by Alef and Nannipieri (1995). Evolved CO_2 was trapped in 0.1 $mol.L^{-1}$ NaOH and quantified by titration with standardized HCl following carbonate precipitation. Results were expressed as µg C–CO_2 per 100 g of dry soil. Measurements were taken at 24 h and at 6, 9, 11, and 15 days of incubation to assess the temporal dynamics of microbial activity in response to the treatments.

Statistical analysis

Chemical and biological variables were analyzed using analysis of variance (ANOVA) under a completely randomized design, following verification of normality (Shapiro–Wilk test) and homogeneity of variances (Levene's test). When significant differences were detected ($p \leq 0.05$), Tukey's honestly significant difference (HSD) test was applied.

Associations between chemical variables and microbial respiration were assessed using Spearman's rank correlation coefficient (ρ), considering $p \leq 0.05$, due to the small sample size (n = 20) and the absence of strict assumptions of normality and linearity. Subsequently, multiple linear regression models were fitted to determine whether the observed associations persisted after controlling for treatment effects, with collinearity evaluated using the Variance Inflation Factor (VIF). Statistical analyses were performed using R software (version 4.3.1) (R Core Team, 2023).

Results and discussion

Soil physical and chemical properties

The evaluated soils exhibited favorable physical conditions in the surface horizon (0–20 cm), with no evidence of compaction-related constraints, and a clay loam texture classification. Moderate variability was observed among samples, particularly in particle-size fractions, reflecting the natural heterogeneity of the study area. Chemically, the soil was characterized by moderately acidic and relatively homogeneous conditions, accompanied by low levels of electrical conductivity (Table 1). Organic carbon content was within the medium to high range for agricultural soils under cold-climate conditions (Osorio, 2012), whereas exchangeable aluminum reached concentrations considered potentially restrictive for root development and microbial activity.

The physical properties of the surface horizon (0–20 cm) characterize a soil typical of conventionally managed high Andean agroecosystems,

Table 1. Descriptive statistics of selected soil physical and chemical properties.

Parameters	Clay (%)	Sand (%)	Silt (%)	GM (%)	BD ($g.cm^{-3}$)	pH (1:2)	EC ($\mu S.cm^{-1}$)	OC (%)	Exch. Al^{3+} ($cmol(+).kg^{-1}$)
Mean	34.62	35.54	29.83	22.54	0.91	5.29	14.39	3.23	2.00
SD	3.29	10.40	7.61	2.87	0.06	0.23	3.09	1.08	0.75
Min	32.30	15.40	23.00	17.01	0.82	5.08	8.70	1.25	0.80
Max	40.75	44.70	43.85	24.62	0.98	5.93	21.50	4.08	3.60
Textural class		Clay loam							

GM: Gravimetric moisture (%); BD: Bulk density (g cm^{-3}); EC = Electrical conductivity; OC: Organic carbon (%); Exch. Al^{3+}: Exchangeable aluminum; SD: Standard deviation; n = 5.

with values indicating the absence of physical limitations for root growth and gaseous diffusion (Osorio, 2012).

The initial soil chemical conditions indicate an environment that is functionally restrictive for microbial respiration, as microbial metabolic activity operates under physiological stress. Low pH reduces enzymatic stability, limits microbial diversity, and decreases carbon use efficiency (Rosinger *et al*., 2025).

Consequently, a greater proportion of the available carbon is released as CO_2 rather than incorporated into biomass, explaining a potentially active yet metabolically inefficient respiratory process (Malik *et al*., 2018).

The presence of exchangeable Al^{3+} further intensifies these limitations by impairing enzymatic activity and exerting selective pressure on microbial communities, favoring groups more tolerant of acidic stress (Ofoe *et al*., 2023).

Variation in soil pH, exchangeable aluminum, and electrical conductivity during soil incubation

At the beginning of the incubation period, significant differences among treatments were detected ($p < 0.05$). The control (T0) exhibited the highest pH value, whereas treatments receiving amendments (T1, T2, and T3) showed no statistically significant differences among them, indicating homogeneous initial conditions within this group.

After 15 days of incubation, statistically significant differences among treatments persisted ($p < 0.05$). Treatment T1 exhibited the highest pH, followed by T2, whereas T3 showed intermediate values, not differing significantly from either T2 or the control. In contrast, T0 consistently presented the lowest pH values.

In the temporal comparison, soil pH increased significantly in the amended treatments. The increase was +0.62 units in T1 (5.20 to 5.82), +0.36 in T2 (5.16 to 5.52), and +0.28 in T3 (5.15 to 5.43). These results demonstrate a dose-dependent effect of $CaCO_3$, with full liming producing the greatest reduction in soil acidity over the evaluated period. In contrast, the control exhibited a significant decrease in pH (−0.30 units), indicating a trend toward increased acidity in the absence of amendment.

Regarding exchangeable Al^{3+}, no significant differences ($p > 0.05$) were detected among treatments either at the beginning or after 15 days of soil incubation. However, in the temporal comparison within each treatment, only T1 exhibited a significant decrease in Al^{3+} (from 2.64 to 1.76 cmol(+).kg^{-1}), whereas T0, T2, and T3 showed no significant changes.

Electrical conductivity (EC), in turn, did not show significant differences among treatments at the beginning of the experiment. However, after 15 days, T1 exhibited significantly lower EC values than T0, T2, and T3, which did not differ from each other. Temporally, all treatments showed significant increases in EC following incubation.

Full liming with $CaCO_3$ (T1) was the only treatment that simultaneously modified both pH and exchangeable Al^{3+} significantly over the evaluated period.

The significant increase in pH observed in the $CaCO_3$ treatments is consistent with the synthesis presented by Bolan *et al.* (2003), who demonstrated that the application of liming materials induces progressive chemical transformations that reduce exchangeable acidity and alter aluminum speciation in the soil.

In the present study, the magnitude of the pH increase was clearly dose-dependent, which is consistent with the findings of Mahmud and Chong (2022), who emphasize that liming efficiency depends not only on the application rate but also on the time available for carbonate reactions in the soil. These authors highlight that the incubation period is critical for achieving a new stable chemical equilibrium, as the reaction of $CaCO_3$ is not instantaneous but rather progressive.

The reduction in exchangeable Al^{3+} observed in the full lime treatment is consistent with the mechanism described by Bolan *et al*. (2003), whereby increasing pH promotes the precipitation of aluminum as less soluble hydroxides and reduces its activity in the exchange complex. However, the less pronounced response in treatments with partial or combined doses suggests that the 15 day period corresponds to an early phase of the neutralization process. In this regard, it has been documented that liming dynamics are progressive and time-dependent; therefore, the reduction in exchangeable aluminum may continue over longer incubation periods as dissolution reactions of the liming material proceed and soil chemical properties gradually stabilize (Wenyika *et al*., 2025).

Regarding the combination of $CaCO_3$ and organic amendments, Giang *et al*. (2024) demonstrated that the incorporation of organic substrates modifies soil organic fractions and biochemical parameters during controlled incubations, influencing mineralization processes and the chemical dynamics of the system.

These results support the interpretation that the organic fraction may contribute to the formation of complexes with Al^{3+}, thereby reducing its mobility and activity in the soil solution, while also enhancing soil buffering capacity (Li *et al*., 2022).

In this regard, organic matter has been shown to play a fundamental role in buffering soil acidity and reducing aluminum leaching in acidic soils. However, these effects tend to manifest more gradually compared to direct chemical liming, due to the progressive nature of organic matter decomposition and the gradual release of reactive compounds (Jiang *et al*., 2018).

Table 2. Effect of amendment treatments on soil pH, exchangeable aluminum, and electrical conductivity at the beginning and after 15 days of incubation.

Treatment	pH (initial, 1:2)	pH (15 days, 1:2)	Exch. Al^{3+} (Initial, cmol(+).kg^{-1})	Exch. Al^{+3} (15 days, cmol(+).kg^{-1})	EC (Initial, µS.cm^{-1})	EC (15 days, µS.cm^{-1})
0	5.64±0.08aA	5.34±0.01cB	1.60±0.36aA	1.68±0.15aA	13.98±0.88aA	49.28±2.26aB
1	5.20±0.04bA	5.82±0.06aB	2.64±0.16aA	1.76±0.16aB	15.64±1.88aA	24.16±2.00bB
2	5.16±0.03bA	5.52±0.02bB	2.16±0.41aA	1.68±0.23aA	13.58±0.86aA	49.64±4.39aB
3	5.15±0.03bA	5.43±0.02bcB	1.60±0.13aA	1.60±0.22aA	14.34±1.86aA	47.28±4.81aB

Values correspond to mean ± standard error (n = 5). Different lowercase letters indicate significant differences among treatments within the same sampling time (Tukey, $p \leq 0.05$), whereas different uppercase letters indicate significant changes between sampling times within each treatment (Tukey, $p \leq 0.05$). Exch. Al3+: exchangeable aluminum; EC: electrical conductivity; T0 = Control; T1 = 100 % de CaCO3; T2 = 50 % de CaCO3; T3 = 50 % CaCO3 + 50 % commercial organo-mineral amendment. Source: Authors.

On the other hand, the significant increase in electrical conductivity following incubation is consistent with the findings of Becerra-Agudelo *et al*. (2022), who report that the application of alkaline amendments alters the distribution of chemical species in the soil and may lead to temporary increases in ion concentrations in solution, associated with the geochemical re-equilibration of the system.

The results of the present study confirm that the efficiency of liming in reducing exchangeable aluminum depends simultaneously on the applied dose and the incubation time. The evaluated period (15 days) allowed for the detection of a significant initial chemical response; however, longer incubation periods may promote more complete aluminum neutralization and the establishment of a more stable chemical equilibrium. This is consistent with recent reports indicating that the effects of liming on aluminum neutralization and soil chemical stabilization are gradual and time-dependent processes (Wenyika *et al*., 2025).

Soil microbial respiration in response to amendment application

Figure 1 shows that cumulative microbial respiration at 24 h of incubation differed significantly among treatments. Amendment treatments (T1, T2, and T3) exhibited significantly higher values than the control (T0). Among them, T1 showed the highest cumulative respiration, whereas T2 and T3 displayed intermediate values, with no significant differences between them.

At 6 days, the same pattern was maintained. T1 exhibited the highest CO_2 accumulation, differing significantly from T0, which showed the lowest values. Treatments T2 and T3 occupied an intermediate position and did not differ significantly from each other, although both exceeded the control.

At 9, 11, and 15 days of incubation, significant differences among treatments persisted. T1 consistently exhibited the highest cumulative microbial respiration across all evaluated time points. Treatments T2 and T3 continued to show intermediate responses and remained statistically similar to each other, whereas T0 presented the lowest values throughout the entire experimental period.

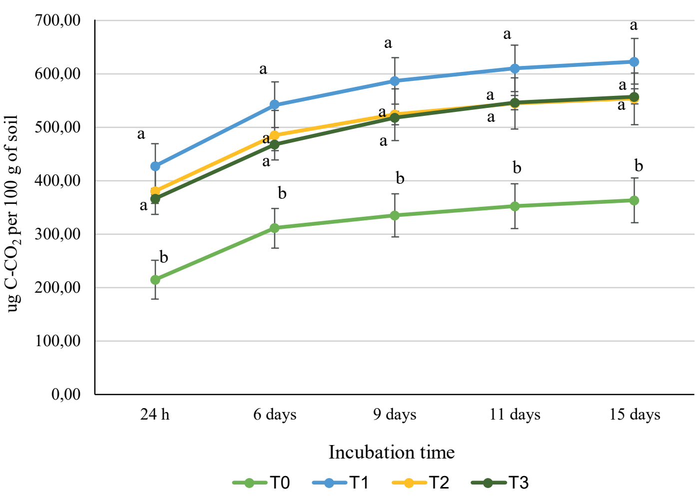

Figure 1. Cumulative microbial respiration (µg $C\text{-}CO_2$.100 g^{-1} of soil) over 15 days of incubation in response to amendment application. Lines represent mean cumulative microbial respiration values for each treatment, and bars indicate the standard error of the mean (± SE; n = 5). Different letters indicate significant differences among treatments within each incubation time (Tukey, $p \leq 0.05$). T0 = Control; T1 = 100 % de $CaCO_3$; T2 = 50% de $CaCO_3$; T3 = 50 % $CaCO_3$ + 50 % commercial organo-mineral amendment. Source: Authors.

The results of the present study are consistent with those reported by Guo *et al*. (2019), who observed that $CaCO_3$ application in acidic soils increased pH and stimulated microbial activity. This increase has been attributed to the reduction of Al^{3+} toxicity and the improvement of chemical conditions regulating microbial metabolism.

In acidic soils, elevated concentrations of exchangeable Al^{3+} can constrain microbial biomass; consequently, liming creates conditions that are potentially more favorable for biological activity (Mitsuta *et al*., 2025).

However, correlation analysis indicated that the association between final pH and respiration was significant only for initial respiration ($\rho = 0.644$; $p = 0.002$), whereas cumulative respiration and respiration at 15 days showed no statistically significant relationships with the evaluated chemical variables. Likewise, exchangeable Al^{3+} showed no significant correlations with respiratory activity ($p > 0.05$). These results suggest that, although pH may modulate microbial responses during the early stages of incubation, it does not persist as an independent predictor of respiratory dynamics over time. This finding is consistent with studies indicating that pH control over microbial carbon processes is context-dependent and mediated by interactions with other edaphic factors (Malik *et al*., 2018).

Multiple linear modeling reinforced this interpretation. After controlling for treatment effects, the associations between pH and respiration were no longer significant, whereas treatment accounted for a substantial proportion of the observed variability ($R^2 = 0.529$; $p = 0.018$ in the reduced model). Consequently, the respiratory response appears to be primarily driven by the integrated effect of the applied management practices rather than by the evaluated chemical variables considered in isolation.

In this context, the greater respiratory increase observed in treatments combining lime with organo-mineral amendments may be attributed to complex interactions among the availability of labile carbon, shifts in microbial community structure, and physicochemical modifications of the soil system. Iticha *et al*. (2026) demonstrated that the interaction between lime and organo-mineral amendments increases the partial pressure of carbon dioxide (pCO_2) and dissolved inorganic carbon, promoting calcite dissolution and accelerating acidity neutralization. This suggests the existence of feedbacks between biological activity and geochemical processes.

Additionally, previous studies have shown that liming can modify the structure and functional stability of the microbial community (Madegwa and Uchida, 2021). In this regard, the increase in cumulative respiration observed in the present study may reflect not only an immediate chemical effect, but also a functional reorganization of the soil microbiome induced by the treatment.

Conclusions

Liming with $CaCO_3$ significantly increased soil pH, reaching increments of up to 0.62 units at the full dose, whereas the control treatment exhibited a decrease of 0.30 units, indicating a dose-dependent effect during the incubation period.

The reduction in exchangeable Al^{3+} was significant only in the treatment with 100 % $CaCO_3$, where it decreased from 2.64 to 1.76 cmol(+).kg^{-1}, with no significant changes observed in the partial or combined dose treatments.

Amendment treatments promoted higher microbial respiration compared to the control throughout the incubation period, with the highest values associated with the full $CaCO_3$ dose.

Overall, multivariate analysis indicated that chemical variables did not independently explain microbial respiration variability, whereas treatment accounted for a significant proportion of the variation ($R^2 = 0.529$; $p = 0.018$).

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