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RESEARCH ARTICLE

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Dolomitic limestone was more effective than calcitic limestone in increasing soil pH in an untilled olive orchard

Margarida Arrobas[1,2] | Nuno Conceição[1,2] | Ermelinda Pereira[1,2] | Sandra Martins[3] | Soraia Raimundo[1,2] | Cátia Brito[3] | Carlos Manuel Correia[3] | Manuel Ângelo Rodrigues[1,2]

[1]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal

[2]Laboratório para a Sustentabilidade e Tecnologia em Regiões de Montanha, Instituto Politécnico de Bragança, Bragança, Portugal

[3]Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes e Alto Douro, Vila Real, Portugal

Correspondence
Margarida Arrobas, Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal.
Email: marrobas@ipb.pt

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Abstract

In acid soils, when no-tillage farmers intend to apply lime, the question arises as to whether it should be incorporated into the soil or whether it can be left on the soil surface. In this study, two types of limestone, calcitic (Lcal) and dolomitic (Lmag), were tested in two olive groves of cv. Cobrançosa, with an initial pH of 4.9 (S. Pedro) and 5.5 (Raparigas). In S. Pedro, limestone was incorporated into the soil (Lburied) and in Raparigas, it was left on the floor (Lfloor). The use of limestone significantly increased soil pH in the 0–0.10 m layer in both experiments. In the 0.10–0.20 m soil layer, only Lmag increased significantly the soil pH in comparison with the control. Lmag was more effective than Lcal in increasing cation exchange capacity (CEC) and reducing exchangeable acidity (EA) and aluminium (Al^{3+}) in the Lfloor experiment. Both limes increased leaf calcium (Ca) concentration, and Lmag increased the leaf levels of magnesium (Mg). In Lfloor experiment (higher soil pH), soil microbial carbon (C) decreased, and microbial nitrogen (N) increased with liming, which may indicate an increase in bacteria in the soil and a decrease in fungi. In Lburied experiment (initial pH of 4.9), liming significantly increased accumulated (2018–2021) olive yield (56 and more than 67 kg tree^{-1}, respectively, in the control and liming treatments). In Lfloor experiment (initial pH of 5.5), the accumulated olive yields did not differ significantly between treatments (average values between 105 and 115 kg tree^{-1}). The results of this study provide evidence that liming may increase olive yield in very acid soils and that dolomitic limestone should preferably be used by no-tillage farmers, due its higher solubility and faster effect on soil and trees.

KEYWORDS

API ZYM assay, leaf nutrient concentration, lime, *Olea europaea*, olive yield, soil properties

1 | INTRODUCTION

Soil acidity is an important ecological factor affecting crop productivity worldwide (Nahar et al., 2022; Wakwoya et al., 2022; Wen et al., 2022). For agricultural purposes, acid soils are considered those that have a pH value of less than 5.5 for most of the year (FAO, 2023). Topsoil acidity affects around 30% of the total ice-free land area of the world, and approximately 50% of the world's potentially arable soils (FAO and ITPS, 2015; Sumner & Noble, 2003).

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Soils can become acid because of natural phenomena, with soil acidity usually occurring in regions where annual precipitation exceeds evapotranspiration and where base cations, such as potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}) and sodium (Na^+), are progressively leached out from soil and replaced by hydrogen (H^+) and aluminium (Al^{3+}; FAO and ITPS, 2015; Holland et al., 2018).

The acidity of agricultural soils can also be induced or exacerbated by continued cultivation. Removal of cations by crops is usually seen as one of the most important anthropogenic causes of soil acidification (Thomas & Hargrove, 2015; Xu et al., 2020), as growing plants uptake more of certain cations (e.g. K^+, NH_4^+, Ca^{2+}, Mg^{2+}) than they do of anions (e.g. NO_3^-, SO_4^{2-}), resulting in the exclusion of H^+ ions in the soil solution to maintain charge balance (Weil & Brady, 2017). Long-term crop fertilizer application may also cause the pH of soils to become more acid (Holland et al., 2019; Thomas & Hargrove, 2015; Xu et al., 2020). Ammonium fertilizers can significantly lower soil pH, since an excess of H^+ ions is generated during the bacterial conversion of NH_4^+ to NO_3^- (Weil & Brady, 2017).

When the pH drops below 5, the solubility of Al increases, providing hostile conditions for root development (Sumner & Noble, 2003). In general, Al toxicity is usually seen as the most important single factor impairing plant growth in acid soils, but manganese (Mn) toxicity and/or Ca, Mg, phosphorus (P) and molybdenum (Mo) deficiencies have also been reported as major causes of the loss of crop productivity (Kochian et al., 2004; Uchida & Hue, 2000; Weil & Brady, 2017).

In mainland Portugal, it is estimated that more than 80% of soils are acidic (Arrobas et al., 2017; Rodrigues et al., 2020). Portugal has a Mediterranean climate and, in most of the territory, annual precipitation does not exceed evapotranspiration (IPMA, 2023). However, in these climates, precipitation is concentrated in the winter months, between October and March, where there is an excess of precipitation over evapotranspiration, which may promote base leaching. Soil acidity is also because of the fact that in a large part of the territory, especially in the north, soils are developed from acidic rocks (Arrobas & Rodrigues, 2020).

A significant part of the world's olive production is found in the Mediterranean basin, where arid and semi-arid climates prevail. In some of these olive-growing areas, the soil pH is neutral to alkaline, but olive is also grown in acid soils. Olive is sometimes referred to as having a very wide pH range, usually from 5.5 to more than 8.0 (Freeman & Carlson, 2005; Parra, 2017). This wide range of soil pH values in which the olive tree can be grown has been attributed to the enormous diversity of cultivated varieties and their varying adaptability to soil pH (Rodrigues & Arrobas, 2013). In the north of Portugal, for instance, it has been reported that olive can grow and produce satisfactorily in very acid soils (pH 5 or less; Lopes et al., 2020, 2022; Rodrigues et al., 2015). However, soil testing laboratories often recommend applying lime for pH values below 6 (INIAV, 2022).

Olive growers across the Mediterranean basin are changing from conventional tillage to different methods of cover cropping (Alcántara et al., 2017; Rodrigues & Arrobas, 2020). This change has important implications for applying limestone, because no-tillage farmers cannot incorporate it into the soil and, therefore, its effect tends to be slower. Although the application of lime in herbaceous crops under no-tillage systems has received due attention (Caires et al., 2008; Flower & Crabtree, 2011; Godsey et al., 2007), the same has not been done for traditional olive orchards. Thus, in this study, we reported the results of two independent experiments, where two types of limestone (calcitic and dolomitic) were applied in olive groves with soils with initial acid pH (4.9 and 5.5). In one olive grove, limestone was incorporated into the soil (Lburied) and in another, it was kept on the surface (Lfloor). The results of the two independent experiments were used to check the hypotheses: (i) the superficial application of limestone is effective and (ii) the use of dolomitic limestone presents greater benefits than calcitic limestone.

2 | MATERIALS AND METHODS

2.1 | Site characterization

The field trial was carried out over 4 years (2018–2021) in two rainfed olive groves located in Raparigas (41°25′09.5″ N, 7°16′14.6″ W; Lfloor) and S. Pedro (41°26′36.2″ N, 7°13′22.6″ W; Lburied), in the municipality of Mirandela, NE Portugal. Lfloor is an olive grove of cv. Cobrançosa, 25 years old, with trees spaced at 7 × 7 m. In recent years, it has been very productive, with olive yields varying between 5 and 6 t ha^{-1}, according to the producer's data, as a result of careful cropping techniques. The Lburied orchard is an olive grove also of cv. Cobrançosa, 18 years old with trees also spaced at 7 × 7 m. At the beginning of the study, these trees displayed a more neglected appearance, as they had not received regular fertilizer application. According to the farmer, olive yield rarely exceeded 1 t ha^{-1}

The regional climate is typically Mediterranean, with two well-marked seasons, a cool winter with some rain and a very hot and dry summer. The annual temperature and precipitation are 14.3°C and 509 mm, respectively. The average monthly air temperature and precipitation recorded during the experimental period are shown in Figure 1.

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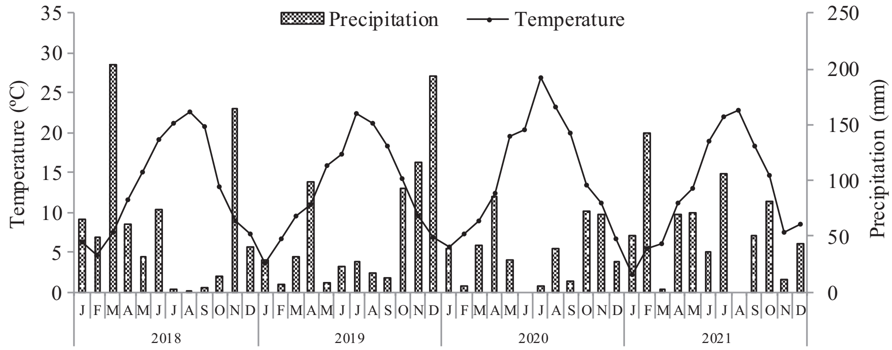
FIGURE 1 Monthly precipitation and average monthly temperature during the experimental period in Mirandela, northeast Portugal.



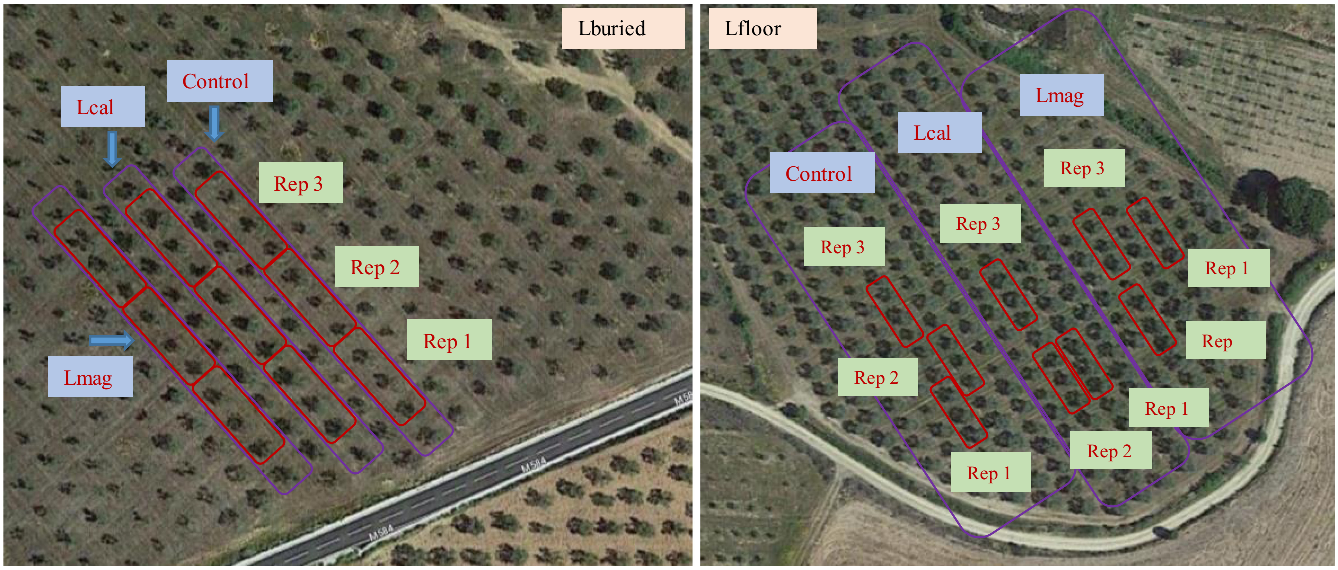


FIGURE 2 Schematic view of the field experiments where limestone (Lmag, dolomitic; Lcal, calcitic) was incorporated into the soil (Lburied) and left on the soil surface (Lfloor).

The soils are Leptosols (WRB, 2015), developed from a bedrock of schist, with an arable layer of barely 0.20 m. At the beginning of the study, in March 2018, a physico-chemical characterization of the soils of the arable layer was carried out from three composite samples taken at a depth of 0–0.20 m. The soil of the Lfloor experiment has a loamy-sand texture (soil separates, 44 g kg^{-1} clay, 207 g kg^{-1} silt and 749 g kg^{-1} sand) and the soil of the Lburied trial a sandy-loam texture (19 g kg^{-1} clay, 238 g kg^{-1} silt, 743 g kg^{-1} sand). The soils of the Lfloor and Lburied orchards are both low in organic C (5.1 and 6.0 g kg^{-1}, respectively), acid (pH$_{H2O}$ 5.5 and 4.9) and with low CEC (3.9 and 4.1 cmol$_c$ kg^{-1}). Other soil properties determined in the initial sampling are presented as Table S1.

2.2 | Experimental designs and orchard management

The experimental protocol included two independent experiments where the same treatments were used: calcitic limestone (Lcal), dolomitic limestone (Lmag) and no lime application (control). Three groups (replications) of three trees (experimental unit) of very similar canopy size were marked. Thus, each treatment was applied to nine trees, with a total of 27 trees marked in each experiment (Figure 2).

Tudicarb®, a commercial product with a granulometry of 0–2 mm and containing 99% calcium carbonate ($CaCO_3$), and a neutralizing value of 96 ($CaCO_3$ equivalent), was applied as calcitic limestone. Tudidol® was used as dolomitic limestone, which has the same granulometry and contains 78% $CaCO_3$, 21% magnesium oxide (MgO) and a neutralizing value of 100 ($CaCO_3$ equivalent). Both limestones were applied to each of the orchards at a rate of 2000 kg ha^{-1}. In Lfloor experiment, the amendments were applied evenly on the soil surface with a centrifugal fertilizer spreader, and they were not incorporated into the soil. This experiment was carried out to represent no-tillage farmers, which are increasingly common in the region. In Lburied experiment, the amendments were applied manually and incorporated into the soil with a field cultivator in the 0–15-cm layer. Between the lines that received limestone a border line was left untreated.

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During the study, all the trees from the two experiments were fertilized in the same way. N, P and K were applied in the form of a compound NPK (15% N, 15% P_2O_5, 15% K_2O) fertilizer at a rate of 1.5 kg (or 30, 7 and 15 kg ha^{-1} of N, P and K, respectively) per tree and year. Boron was applied as borax (11% B) at a rate of 1.5 kg ha^{-1} year^{-1} of B. The fertilizers were applied every year late March. The soil surface was kept with a cover of natural vegetation during the winter period. In early spring, the weeds were killed with glyphosate [N-(phosphonomethyl) glycine, 360 g L^{-1} of active ingredient], a postemergence herbicide, applied at a rate of 3 L ha^{-1}. During the experimental period, the orchards received light pruning every year as a way of maintaining similar canopies over the years. It was not necessary to apply pesticides during the study since no relevant phytosanitary problems were observed.

2.3 | Field and laboratory determinations

The olive yields were recorded for four consecutive harvests from November 2018 to November 2021. The trees were harvested by a trunk shaker head, which detaches the olives that are thereafter recovered with an associated inverted umbrella system. After that, sheets were spread on the ground to receive the fruits so that they could be weighed individually per groups of three trees (the experimental unit).

Twice a year, in the summer (during endocarp sclerefication) and in the resting period of winter, leaf samples were taken for elemental analysis. Young fully expanded leaves were collected from the middle of nonbearing current season shoots of the four quadrants around the tree canopy. The samples of leaves were oven-dried at 70°C at constant weight and ground. The elemental composition of leaf samples was determined by the Kjeldahl (N), colorimetry (B and P) and atomic absorption spectrophotometry [K, Ca, Mg, Mn, iron (Fe), copper (Cu), zinc (Zn)] methods (Temminghoff & Houba, 2004) after tissue samples had been digested with nitric acid in a microwave.

The soil was sampled in March 2018, at the beginning of the experiments, for an initial characterization of the plots at 0–0.20 m (Table S1). A second soil sampling was performed in December 2021, at the end of the experiments, to assess the effect of the treatments on soil properties. For this analysis, the samples were taken separately in two layers (0–0.10 m and 0.10–0.20 m). Each soil sample was prepared as a composite sample by collecting and mixing soil from nine points in each of the three replicates (three points per tree).

The dried (40°C) and sieved (2-mm mesh) soil samples were submitted to the following analytical determinations: (1) pH (H_2O and KCl) (by potentiometry); (2) organic C (Walkley–Black method); (3) total N (Kjeldahl); (4) exchangeable bases (ammonium acetate, pH 7.0); (5) extractable P and K (ammonium lactate solution at pH 3.7, Egner–Riehm method); (6) extractable B (hot water and azomethine-H method); and (7) extractable Fe, Mn, Zn, Cu (ammonium acetate and EDTA, determined by atomic absorption spectrometry). In the initial samples, (8) clay, silt and sand fractions were also determined (Robinson pipette method). Methods 1–4 and 6–8 are fully described by Van Reeuwijk (2002) and Method 5 by Balbino (1968).

Soil microbial biomass C (Mic-C) and N (Mic-N) were determined for fresh samples, using the chloroform fumigation–extraction method (Vance et al., 1987), after 24 h of conditioning at 25°C and 60% water holding capacity. Organic C and total soluble N were determined by near infrared detection (NIRD) and by chemiluminescence detection after combustion at 850°C in an elemental analyzer (Formac, Skalar). The Mic-C and Mic-N were calculated using a KEC factor of 0.33 (Vance et al., 1987) and KEN factor of 0.54 (Brookes et al., 1985), respectively. All results are expressed on an oven-dry (105°C) weight basis.

Enzymatic activity in soil samples (0–0.10 m) was determined by the API ZYM strip system (BioMerieux). It consists of a semi-quantitative analysis of 19 enzymatic reactions, including three phosphatases (alkaline phosphatase, acid phosphatase and phosphohydrolase), three esterases (lipase, esterase–lipase and esterase), three aminopeptidases (leucine amino-peptidase, valine amino-peptidase and cystine aminopeptidase), two proteases (chymotrypsin and trypsin) and eight glycosyl-hydrolases (-galactosidase, -glucosidase, N-acetyl-glucosaminidase, -glucosidase, -galactosidase, -glucuronidase, -mannosidase and -fucosidase). The API ZYM strips are composed of 20 microcupules containing dehydrated chromogenic substrates for 19 enzymatic reactions and a control that does not contain any enzyme substrate. After the laboratory preparation of the test, a semi-quantitative evaluation of the enzymatic activities was measured by reference to a colorimetric standard table by assigning numerical values of 0–5, where 0, 1, 2, 3, 4 and 5 are, respectively, 0, 5, 10, 20, 30 and 40 or more nanomoles of substrate hydrolyzed. In this study, the intensity of enzymatic reactions in each microcupule was measured by four independent people, and the final result was the average of their records. For a more detailed description of this methodology, the reader is referred to Martínez et al. (2016).

2.4 | Data analysis

Data were tested for normality and homogeneity of variances using the Shapiro–Wilk test and Bartlett's test,

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respectively. A comparison of the effect of the fertilizer treatments in each field experiment was provided by one-way ANOVA. When significant differences were found ($p < .05$), the means were separated by the multiple range Tukey's HSD test ($\alpha = 0.05$).

For the most responsive variables to liming, including olive yield, leaf minerals, soil properties and enzyme activity, a principal component (PC) analysis (PCA) was carried out in the correlation matrix PCA and PC's, with eigenvalue above 1 selected. Monotonic relationships between variables were investigated and quantified by the Spearman's correlation coefficient (rs, $\alpha = 0.05$). Computations were carried out with an open source statistical software (R Core Team, 2022).

3 | RESULTS

3.1 | Soil properties

In the 0–0.10-m soil layer, $pH_{(H2O, KCl)}$ values were significantly lower in the control than in the liming treatments in both experiments (Table 1). In the Lburied experiment, soil $pH_{(H2O)}$ was significantly higher in the Lmag than in Lcal treatment. Extractable P did not vary significantly between treatments in Lfloor and was significantly lower in the control than in the limed plots in Lburied experiment. Extractable K did not vary between treatments in any of the experiments. Exchangeable Ca^{2+} and Mg^{2+} were significantly lower in the control in comparison with the liming treatments in both orchards. In Lfloor experiment, Lmag showed significantly higher exchangeable Mg^{2+} than the control and Lcal. The values of exchangeable K^+ and Na^+ did not vary significantly between treatments in both orchards. The EA was significantly lower in Lcal and Lmag than in the control. In Lburied experiment, CEC varied significantly between treatments in both experiments, with the liming treatments showing higher values than the control. Soil B, Cu, Zn, Fe and Mn levels did not vary significantly between treatments, and the results were presented as Table S2.

In the 0.10–0.20-m soil layer, the values of $pH_{(H2O)}$ and $pH_{(KCl)}$ were significantly higher in the Lmag treatment than in the control, in both the Lfloor and Lburied experiments (Table 2). The pH values for the Lcal treatment were between those of Lmag and the control without significant differences for any of them. P values were significantly higher in the Lmag treatment than in the control. Exchangeable Ca^{2+} did not vary significantly between treatments in the 0.10–0.20-m soil layer in the Lfloor experiment, whereas exchangeable Mg^{2+} was significantly higher in the Lmag treatment than in the other treatments. In Lburied experiment, the results followed the trend reported for the 0–0.10-m layer. The EA and

TABLE 1 Soil properties from samples taken in December 2021 at a depth of 0–0.10 m in the orchards where lime (calcitic, Lcal; dolomitic, Lmag) was left on the floor (Lfloor) and incorporated into the soil (Lburied).

	Lfloor			Lburied		
	Control	Lcal	Lmag	Control	Lcal	Lmag
pH (H_2O) (1:2.5)[a]	5.60 B	6.29 A	6.25 A	4.71 c	5.59 b	5.68 a
pH (KCl) (1:2.5)[a]	4.22 B	5.19 A	5.11 A	3.65 b	4.80 a	4.82 a
P (mg_5 kg^{-1}, P_2O_5)[b]	9.3 A	11.9 A	9.8 A	52.1 b	111.6 a	96.9 a
K (mg kg^{-1}, K_2O)[b]	175.0 A	160.0 A	138.3 A	458.3 a	585.0 a	538.0 a
Ca^{2+} ($cmol_c$ kg^{-1})[c]	1.97 B	3.80 A	2.98 A	1.00 b	5.48 a	4.78 a
Mg^{2+} ($cmol_c$ kg^{-1})[c]	0.91 B	0.66 B	1.91 A	0.37 b	0.88 a	0.99 a
K^+ ($cmol_c$ kg^{-1})[c]	0.35 A	0.35 A	0.27 A	1.06 a	1.21 a	1.22 a
Na^+ ($cmol_c$ kg^{-1})[c]	0.13 A	0.13 A	0.13 A	0.36 a	0.73 a	0.77 a
EA ($cmol_c$ kg^{-1})[d]	0.33 A	0.23 A	0.30 A	0.87 a	0.20 b	0.23 b
Al^{3+} ($cmol_c$ kg^{-1})[e]	0.20 A	0.10 A	0.07 A	0.80 a	0.17 b	0.22
CEC ($cmol_c$ kg^{-1})[f]	3.70 B	5.17 AB	5.59 A	3.65 b	8.51 a	8.06 a

Note: By application method, means followed by the same letter (capitals in Lfloor and lowercase in Lburied) are not significantly different by Tukey's HSD test ($\alpha = 0.05$).

[a]Potentiometry.

[b]Extractable phosphorus and potassium (ammonium lactate).

[c]Extractable calcium, magnesium, potassium and sodium (ammonium acetate).

[d]Exchangeable acidity.

[e]Aluminium (potassium chloride).

[f]Cation exchange capacity.

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TABLE 2 Soil properties from samples taken in December 2021 at a depth of 0.10–0.20 m in the orchards where lime (calcitic, Lcal; dolomitic, Lmag) was left on the floor (Lfloor) and incorporated into the soil (Lburied).

	Lfloor			Lburied		
	Control	Lcal	Lmag	Control	Lcal	Lmag
pH (H_2O) (1:2.5)[a]	5.16 B	5.40 AB	5.82 A	4.46 b	5.04 ab	5.45 a
pH (KCl) (1:2.5)[a]	3.75 B	4.13 AB	4.57 A	3.71 b	4.32 ab	4.88 a
P ($mg\,kg^{-1}$, P_2O_5)[b]	6.5 A	6.6 A	6.9 A	25.2 b	29.7 ab	54.8 a
K ($mg\,kg^{-1}$, K_2O)[b]	92.0 A	59.0 A	73.3 A	295.0 a	277.3 a	311.7 a
Ca^{2+} ($cmol_c\,kg^{-1}$)[c]	1.50 A	1.60 A	1.81 A	0.73 b	3.56 a	4.71 a
Mg^{2+} ($cmol_c\,kg^{-1}$)[c]	0.66 B	0.58 B	1.47 A	0.38 b	0.86 a	0.68 a
K^{+} ($cmol_c\,kg^{-1}$)[c]	0.17 A	0.18 A	0.18 A	0.47 a	0.45 a	0.75 a
Na^{+} ($cmol_c\,kg^{-1}$)[c]	0.13 A	0.13 A	0.13 A	0.37 ab	0.82 a	0.18 b
EA ($cmol_c\,kg^{-1}$)[d]	0.83 A	0.73 A	0.30 B	1.23 a	0.73 ab	0.27 b
Al^{3+} ($cmol_c\,kg^{-1}$)[e]	0.57 A	0.47 A	0.13 B	1.07 a	0.63 ab	0.23 b
CEC ($cmol_c\,kg^{-1}$)[f]	3.29 A	3.22 A	3.90 A	3.19 b	6.42 a	6.58 a

Note: By application method, means followed by the same letter (capitals in Lfloor and lowercase in Lburied) are not significantly different by Tukey's HSD test ($\alpha = 0.05$).

[a]Potentiometry.

[b]Extractable phosphorus and potassium (ammonium lactate).

[c]Extractable calcium, magnesium, potassium and sodium (ammonium acetate).

[d]Exchangeable acidity.

[e]Aluminium (potassium chloride).

[f]Cation exchange capacity.

TABLE 3 Organic carbon (OC), total nitrogen (TN), C/N ratio, microbial biomass C (Mic-C) and microbial biomass N (Mic-N) from samples taken in December 2021 at a depth of 0–0.10 m in the orchards where lime (calcitic, Lcal; dolomitic, Lmag) was left on the floor (Lfloor) and incorporated into the soil (Lburied).

	Lfloor			Lburied		
	Control	Lcal	Lmag	Control	Lcal	Lmag
OC ($g\,kg^{-1}$)	7.29 A	7.28 A	6.86 A	9.75 a	9.85 a	8.46 a
TN ($g\,kg^{-1}$)	0.77 A	0.77 A	0.78 A	1.01 a	0.88 a	0.89 a
C/N ratio	9.49 A	9.50 A	8.94 A	9.69 a	11.30 a	9.77 a
Mic-C ($mg\,kg^{-1}$)	216.7 A	162.5 A	61.1 B	91.7 b	167.7 a	177.9 a
Mic-N ($mg\,kg^{-1}$)	18.1 B	18.3 B	34.1 A	20.2 a	5.6 b	2.2 b

Note: By application method, means followed by the same letter (capitals in Lfloor and lowercase in Lburied) are not significantly different by Tukey's HSD test ($\alpha = 0.05$).

Al^{3+} varied significantly between treatments in the Lfloor experiment with significantly lower values recorded in the Lmag treatment. In Lburied experiment, the results followed again the trend of the 0–0.10-m layer. The CEC, highly influenced by Ca^{2+}, did not vary significantly between treatments in the 0.10–0.20-m soil layer in the Lfloor experiment.

The organic C and total (Kjeldahl) N content in the soil, as well as the C/N ratio, did not vary significantly between treatments in any of the experiments (Table 3). The microbial C was significantly lower in the Lmag treatment than in the other treatments in the Lfloor experiment, and significantly higher in the two liming treatments in Lburied experiment. In turn, microbial N varied exactly in the opposite direction to microbial C.

3.2 | Leaf nutrient concentration

In the Lfloor experiment, the concentration of Ca in the leaves was significantly different between treatments on three of the seven sampling dates, with the values of the control treatment remaining below the values of the liming treatments (Figure 3). In the Lburied experiment,

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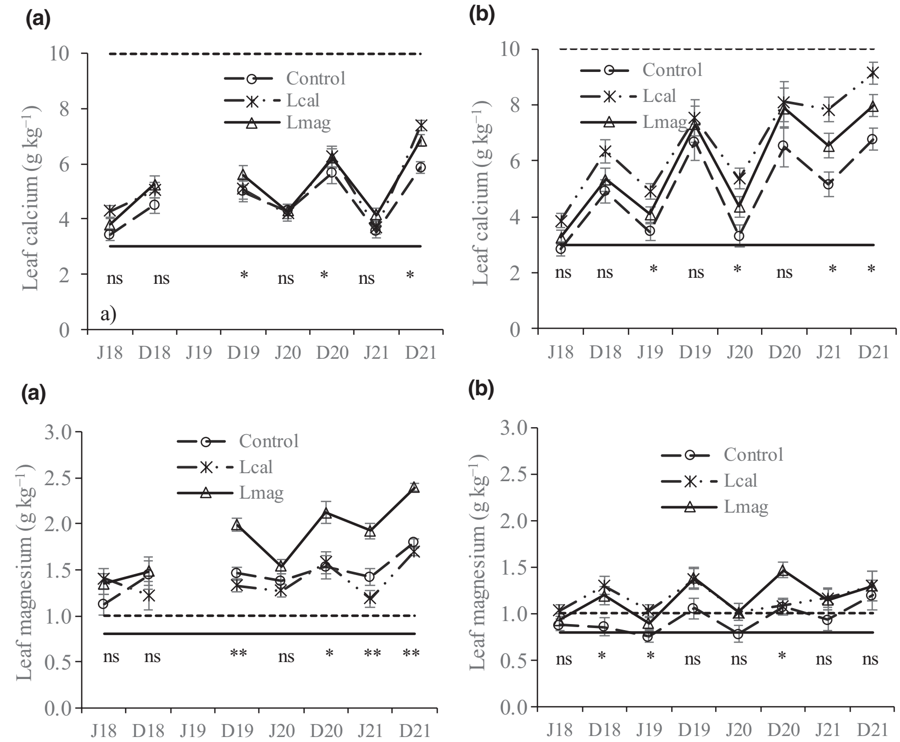

FIGURE 3 Leaf calcium and magnesium concentrations during eight sampling dates from July 2018 (J18) to December 2021 (D21) in the experiments (a) where lime was left of the floor and (b) incorporated into the soil. ns, not significant; *, ** significant at $p < .05$ and $p < .01$, respectively. Vertical line segments are the standard errors. Dashed and solid lines are, respectively, the lower limit of the adequate range and the deficiency threshold for summer sampling after Fernández-Escobar (2017).

there were significant differences between treatments in the leaf Ca concentrations on four of the eight sampling dates. In this trial, the mean values of the control tended to be further away from those of the fertilized treatments. Ca concentrations in leaves showed marked differences between winter and summer samples in both experiments. The summer values of the control treatment approached threshold deficiency, more notably in the Lburied experiment. Also, in the Lburied experiment, Ca levels seemed to increase over time, including in the control treatment, maybe because of the fact that this olive grove is not usually fertilized and is now receiving a compound NPK fertilizer that, in addition to N, P and K, also provides some Ca.

In the Lfloor treatment, Mg levels in the leaves differed significantly between treatments on four of the seven sampling dates (Figure 3). In this experiment, the Mg concentrations in the leaves were higher in the Lmag than in the other treatments. In Lburied trial, there were significant differences on three of the eight sampling dates and, although not always consistently, the Mg concentrations in the leaves tended to be higher in the Lmg treatment. In general, the levels of Mg in the leaves were lower in the Lburied than in that of Lfloor experiment.

It was also reported the concentration in the leaves of N and P, because of their importance in plant nutrition, but also of Mn, because of its relationship with soil pH (Figure 4). The concentrations of other nutrients in the leaves, such as K and the micronutrients B, Fe, Zn and Cu, were little influenced by the treatments and presented as Figure S1.

Leaf N concentrations did not show significant differences between treatments on any of the sampling dates in the Lfloor experiment (Figure 4). The values were above the lower limit of the sufficiency range on all sampling dates. In Lburied experiment, leaf N concentrations showed significant differences between treatments only in one of the eight leaf samplings. On the sampling date where significant differences were found, the higher values were observed in the Lmag treatment. However, this result is not consistent with the other sampling dates, where it seems that the treatments did not have a relevant effect on the N concentration in the leaves.

P concentration in leaves did not differ significantly between treatments on any of the sampling dates in the Lfloor experiment (Figure 4). The values remained low, but generally above the lower limit of the sufficiency range. In Lburied experiment, the values were very low, sometimes being below the lower limit of the sufficiency range, but also without significant differences between treatments, with the exception of the last sampling date, in which the control showed lower values than liming treatments. In Lburied experiment, there was observed an increasing trend over time for P concentrations in leaves, as a direct result of the application of the NPK fertilizer, since this olive grove did not usually receive fertilizers.

In the Lfloor experiment, the Mn concentration in the leaves differed between treatments on three of the seven sampling dates (Figure 4). The values of the Lmag treatment tended to be higher than those of the other treatments. In Lburied experiment, leaf Mn concentrations in

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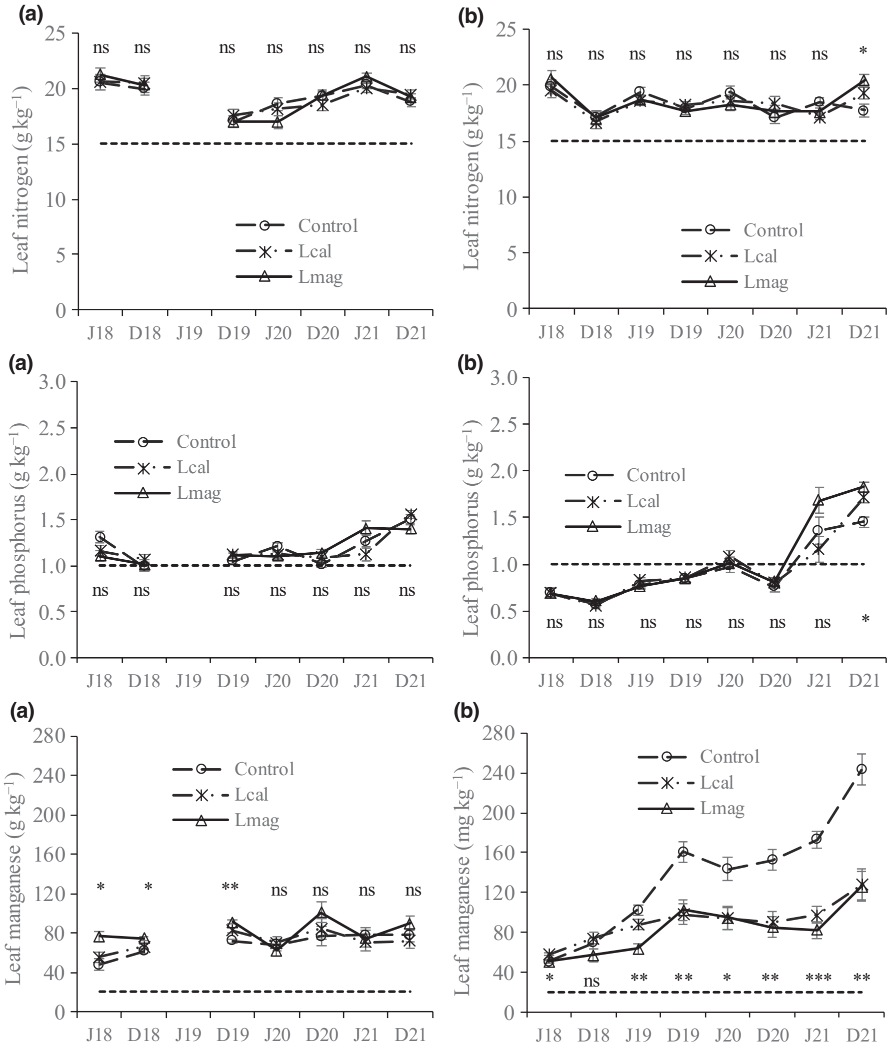

FIGURE 4 Leaf nitrogen, phosphorus and potassium concentrations during eight sampling dates from July 2018 (J18) to December 2021 (D21) in the experiments (a) where lime was left of the floor and (b) incorporated into the soil. ns, not significant; *, ** significant at $p<.05$ and $p<.01$, respectively. Vertical line segments are the standard errors. Dashed lines are the lower limit of the adequate range for summer sampling, after Fernández-Escobar (2017).

the control were significantly higher than in the liming treatments, being notably consistent from the third sampling date, and with values showing an increasing trend over time.

3.3 | Soil enzyme activity

The API ZYM system provides a semi-quantitative analysis of 19 enzymatic reactions. However, for many, no relevant activity or significant differences between treatments (cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, α-mannosidase, α-fucosidase, N-acetyl-ß-glucosaminidase, leucine arylamidase) were recorded in any of the soils (Table 4). Some enzymatic reactions, which are related to soil pH, showed increased activity in more acid (acid phosphatase, naphthol-AS-BI-phosphohydrolase, valine arylamidase, β-glucosidase) or alkaline (alkaline phosphatase) soils. Others did not seem to be pH-dependent, but showed significant differences between treatments (esterase lipase, lipase).

3.4 | Olive yield

In the Lfloor experiment, the application of limestone, calcitic or dolomitic, did not increase the annual olive yields in the first 3 years of the experiment or the accumulated olive yield after the 4 years of testing (values varying between 105.6 and 114.9 kg tree^{-1}; Figure 5). However, in the last year (2021), the control treatment produced significantly less than the Lmag treatment and showed a lower mean value than the Lcal treatment. As significant differences occurred only in the last year, they may indicate a slow effect of limestone when they are not incorporated. These results can also reveal a more effective effect of dolomitic limestone under these conditions.

In the Lburied experiment, there were significant differences between treatments in the third year of the trial, with the values of the Lcal treatment being significantly higher than those of the control treatment. The accumulated olive yields were significantly higher in liming treatments (69.8 and 76.8 kg tree^{-1}, in Lmag and Lcal, respectively) in comparison with the control (55.3 kg tree^{-1}).

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TABLE 4 Enzyme activity measured in soil samples taken at 0–0.10 m in December 2021 in the orchards where lime (calcitic, Lcal; dolomitic, Lmag) was left on the floor (Lfloor) and incorporated into the soil (Lburied) using API ZYM assay.

	Lfloor			Lburied		
Enzyme	**Control**	**Lcal**	**Lmag**	**Control**	**Lcal**	**Lmag**
Alkaline phosphatase	2.8 A	2.3 A	2.3 A	0.9 c	2.3 b	3.0 a
Esterase Lipase (C 8)	3.5 A	2.3 B	2.0 B	2.8 b	2.5 b	3.3 a
Lipase (C 14)	1.3 A	0.8 B	0.4 C	0.6 a	0.8 a	0.5 a
Leucine arylamidase	1.6 A	1.9 A	1.6 A	2.1 ab	1.8 b	2.5 a
Valine arylamidase	1.3 A	1.2 AB	0.7 B	2.9 a	2.0 c	2.5 b
Cystine arylamidase	0.8 A	0.7 A	0.1 B	0.0 a	0.0 a	0.3 a
Trypsin	0.0 A	0.0 A	0.0 A	0.3 a	0.0 a	0.3 a
α-chymotrypsin	0.3 A	0.0 A	0.0 A	nd	nd	nd
Acid phosphatase	4.9 A	3.9 B	3.5 B	3.4 a	2.8 a	3.0 a
Naphthol-AS-BI-phosphohydrolase	4.1 A	3.6 B	3.5 B	4.0 a	3.3 b	3.5 b
α-galactosidase	0.3 A	0.0 B	0.0 B	0.2 a	0.5 a	0.5 a
β-galactosidase	0.3 A	0.0 B	0.0 B	0.2 a	0.8 a	0.8 a
β-glucuronidase	0.2 A	0.0 A	0.0 A	nd	nd	nd
α-glucosidase	0.6 A	0.4 A	0.0 A	0.0 a	0.3 a	0.0 a
β-glucosidase	1.4 A	0.3 B	0.1 B	2.1 a	1.9 a	2.0 a
N-acetyl-ß-glucosaminidase	0.2 A	0.0 A	0.0 A	1.5 a	0.4 b	1.5 a
α-mannosidase	0.3 A	0.2 A	0.1 A	nd	nd	nd
α-fucosidase	0.3 A	0.1 A	0.0 A	nd	nd	nd

Note: By method of application, means followed by the same letter (capitals in Lfloor and lowercase in Lburied) are not significantly different by Tukey's HSD test ($\alpha = 0.05$).

Abbreviation: nd, not detected.

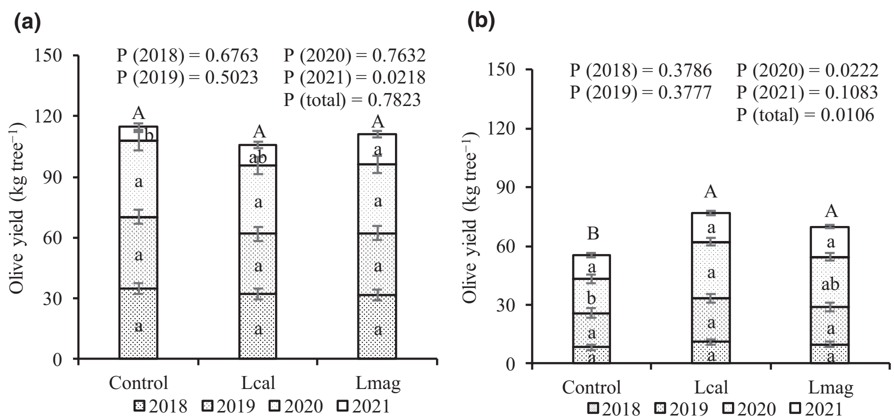

FIGURE 5 Olive yields during four consecutive years and accumulated total in the orchards (a) where lime (calcitic, Lcal; dolomitic, Lmag) was left on the floor and (b) incorporated into the soil. By year (lowercase) and total (uppercase), mean olive yields followed by the same letter are not significantly different by Tukey HSD test ($\alpha = 0.05$). Error bars are the standard errors.

3.5 | Principal component analysis

From the Lfloor experiment, the higher absolute rs values were observed for the variables Mic.C with Lipase (0.95), Soil.pH with Soil.CEC (0.95) and Esterase and Acid.phos (0.91; Table 5). Negative relationships were found to Naph.phos with Soil.CEC (−0.84) and with Soil.pH (−0.80), and to Mic.N with Lipase (−0.80). The biological variables Naph.phos, Acid.phos, Esterase, Lipase, Mic.C and Mic.N were all involved in relationships with many others. From the Lburied experiment, there were found 10 relationships with absolute rs values between 0.80 and 0.92. Negative values lower than −0.80 were found for 13 relationships. Mic.N (5), Soil.CEC, Soil.P, Naph.phos and Val.aryl (4) and Yield (3) they were the ones that were involved in more correlations, among themselves and with other variables. Considering the two experiments, the variables involved in more correlations, positive or negative,

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TABLE 5 Correlation matrices (Spearman's rank correlation coefficient, rs) estimated from data taken in December 2021, separately for the experiments where lime was left on the floor (Lfloor) and incorporated into the soil (Lburied).

<table>
<tr><th></th><th>Yield</th><th>Mic.C</th><th>Mic.N</th><th>Leaf Mn</th><th>Leaf P</th><th>Leaf Ca</th><th>Leaf Mg</th><th>Soil pH</th><th>Soil P</th><th>Soil Ca</th><th>Soil Mg</th><th>Soil CEC</th><th>Alk. Phos.</th><th>Esterase</th><th>Lipase</th><th>Val. Aryl</th><th>Acid. Phos.</th><th>Naph. Phos</th><th>ß. gluco</th></tr>
<tr><td>Yield</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Mic.C</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Mic.N</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Leaf Mn</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Leaf P</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Leaf Ca</td><td>|0.82</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Leaf Mg</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Soil pH</td><td></td><td></td><td>|−0.86</td><td></td><td>|0.83</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Soil P</td><td></td><td></td><td></td><td>|−0.83</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Soil Ca</td><td></td><td></td><td>|−0.83</td><td></td><td></td><td></td><td></td><td></td><td>|0.80</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Soil Mg</td><td></td><td>|0.88</td><td>|−0.92</td><td></td><td></td><td></td><td>0.88|</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Soil CEC</td><td></td><td></td><td>|−0.95</td><td></td><td></td><td></td><td></td><td>0.95|</td><td>|0.83</td><td>|0.87</td><td>|0.92</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Alk.phos.</td><td></td><td>|0.80</td><td>|−0.82</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>|0.86</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Esterase</td><td></td><td>0.87|</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Lipase</td><td></td><td>0.95|</td><td>−0.80|</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>0.83|</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Val.aryl</td><td>|−0.88</td><td></td><td></td><td></td><td></td><td>|−0.83</td><td></td><td></td><td>|−0.81</td><td>|−0.91</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Acid.phos.</td><td></td><td>0.84|</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>0.91|</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Naph.phos</td><td>|−0.83</td><td>0.88|</td><td></td><td></td><td></td><td>|−0.94</td><td></td><td>−0.80|</td><td>|−0.80</td><td></td><td></td><td>−0.84|</td><td></td><td></td><td>0.87|</td><td>|0.91</td><td>0.81|</td><td></td><td></td></tr>
<tr><td>ß.gluco</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>0.88|</td><td></td><td></td><td>0.87|</td><td></td><td></td></tr>
</table>

Note: The variables (with $n = 9$ observations) refer to soil chemical and enzymatic properties (0–0.10 m), leaf nutrient concentration and accumulated olive yield. In each table entry, the left and right characters (relative to the vertical bar) refer to Lfloor and Lburied experiments, respectively. Only significant rs values ($\alpha = 0.01$) were printed.

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were Naph.phos (9), Mic.C and Mic.N (6), Soil.CEC (5), Soil.P and Val.aryl (4). Yield, in turn, was involved in three, with Leaf.Ca (0.82), Val.aryl (−0.88) and Naph. phosp (−0.83).

The first two PC's retained 88% of the total variance in the data set of both experiments, being that the first PC (second PC) explained 73% (15%) in Lfloor and 77% (11%) in Lburied experiments (Figure 6). Considering that a variable with squared cosine above 0.75 (var.cos2 >= 0.75) as having high correlation with a PC, and enumerating only these, for PC1 in Lfloor, Lipase, Acid.phos, Mic.C, Esterase and Naph.phos were positively correlated, whereas for Lburied experiment the variables positively correlated were Soil.Ca, Soil.CEC, Soil.pH, Soil. Mg, Mic.C and Soil.P. Leaf. Mn, Naph.phos, Mic.N and Val.aryl were, in turn, negatively correlated. For PC2 (Lfloor), the variables with contribution above 12% (expected mean, 1/11) showed cos2 from 0.4 to 0.2 were Leaf. Mg, Soil. Mg and Mic.N with positive correlations and Soil.pH with negative correlation. For Lburied, variables whose contribution is above 16% and cos2 from 0.37 to 0.26 were Alk.phos with positive correlation and Yield and Alk.phos with negative correlation.

From the biplots for PC1 and PC2 (Figure 6), it stood out that is possible to identify, for the two experiments, three groups of observations corresponding to the experimental treatment (Control, Lmag and Lcal). However, for Lburied experiment, one observation from Lcal needs to be considered an exception because it is positioned very close to Lmag point cloud. For Lfloor experiment, Control and Lmag groups are positioned in the first and second quadrant of the correlation circle, respectively, while Lcal points are positioned in the negative side of PC2 axis. Control, Lmag and Lcal can be associated, respectively, to higher, lower and intermediate values for the biological variables Lipase, Acid. phos, Mic.C, Esterase and Naph.phos. For Soil.pH variable, Lcal and also Lmag and Control can be associated with higher and lower values, respectively. Lmag had the higher values for the variables Mic.N, Leaf. Mg and Soil. Mg. For the Lburied experiment, Group 1 (G1, first quadrant) include all observations from Lmag treatment and one observation from Lcal treatment; Group 2 (G2, left side of PC1 axis) coincide with Control; and Group 3 (G3, fourth quadrant) include the renaming two observations from Lcal treatment. G2 can be associated with the higher values of Leaf. Mn and Naph.phos (and Val. aryl and Min.N), and G1 and G3 with the lower values. For the variables Yield and Leaf.Ca, the groups G3 and G1 can be associated with the higher and the lower values, respectively. G2 can be associated with the lower values of Soil.Ca, Soil.CEC, Soil.pH, Soil. Mg, Mic.C

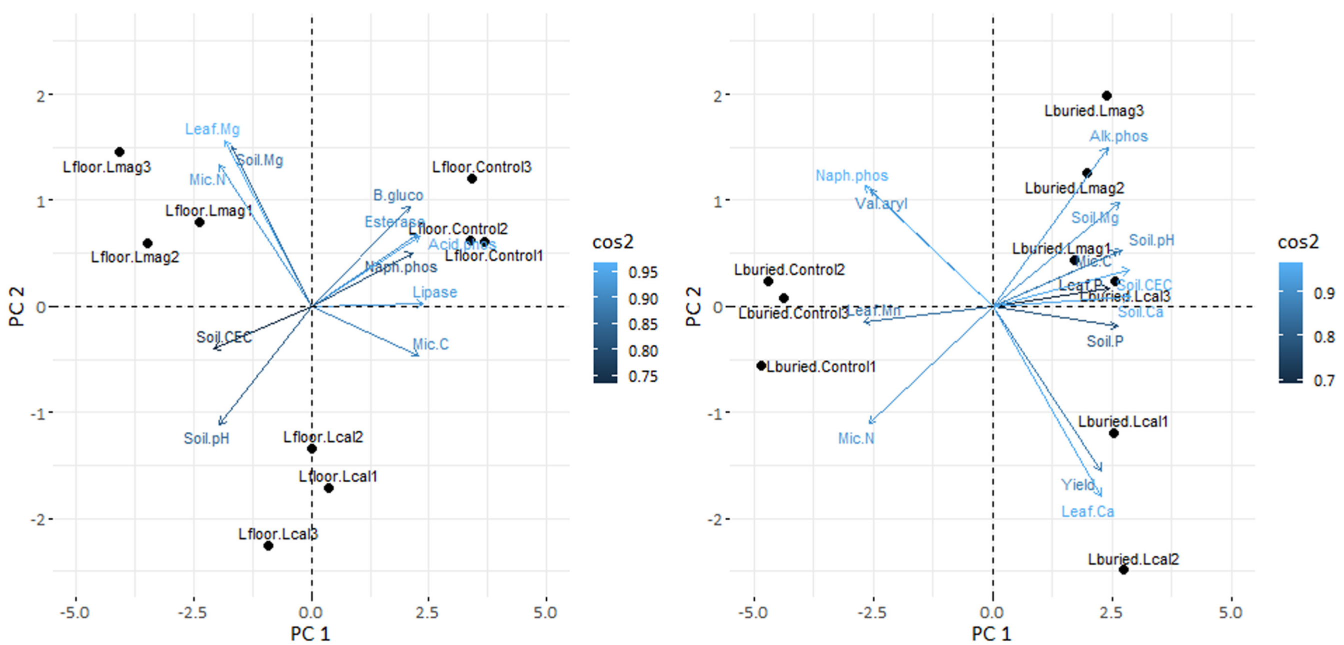

FIGURE 6 Biplot for the plane defined by the two first principal components (PC1 and PC2) resulting from the correlation matrix PCA calculated using only variables to whom Spearman's rank correlation coefficient (rs) was considered statistically significant ($\alpha = 0.01$). Data taken in December 2021, separately for the experiments where lime was left on the floor (Lfloor) and incorporated into the soil (Lburied), and using variables related to soil chemical and enzymatic properties (0–0.10 m), leaf nutrient concentration and accumulated olive yield. Panel (a) and (b) PCA analysis for Lfloor and Lburied experiments, respectively. The considered variables (11 for Lfloor and 14 for Lburied) were represented by vectors. The biplot legend refers to var.cos2. The (symbols) points represent the experimental treatments [Control, calcitic (Lcal) and dolomitic (Lmag) limestone, $n = 3$].

and Soil.P, while G1 and G3 can be associated with the higher values.

4 | DISCUSSION

The application of calcitic or dolomitic limestone increased the soil pH in the 0–0.10 m layer in comparison with the control in both experiments. Increasing pH is the main purpose of applying lime (Cai et al., 2022; Holland et al., 2019; Nahar et al., 2022). After limestone is applied, the pH rises because of reactions with CO_2 from the soil atmosphere and water, forming hydrogencarbonate that neutralizes the exchangeable and residual soil acidity, releasing CO_2 into the atmosphere. The Ca and Mg hydrogencarbonates are much more soluble than the carbonates in the lime are, so acid ions (H^+, Al^{3+}, Mn^{2+}) in the soil solution, and adsorbed on soil colloids, exchange with the basic cations (Ca^{2+} and Mg^{2+}) and precipitate, leading to a rise in pH (Juo & Franzluebbers, 2003; Weil & Brady, 2017).

The results showed that dolomitic limestone was more effective in raising the soil pH than calcitic limestone, since the $pH_{(H2O)}$ in the 0–0.10-m layer was significantly higher in the Lmag than in the Lcal treatment in Lburied experiment, and showed a clear trend towards higher values in the 0.10–0.20-m layer in Lfloor experiment. The dolomitic limestone used in this study had a slightly higher neutralizing value (100, $CaCO_3$ equivalent) than the calcitic limestone (96, $CaCO_3$ equivalent), with the neutralizing value being the main cause determining the extent of the rise in pH (Upjohn et al., 2005). However, in this study, the small difference in neutralizing value of the two limestones will not have had a predominant effect. Dolomitic limestone is more soluble than calcitic limestone, thus acting faster (Cifu et al., 2004; Juo & Franzluebbers, 2003). This may have been the main reason for the most evident effect on the rise in the pH of the dolomitic limestone, especially in Lfloor where the amendments were not incorporated into the soil.

The higher levels of Mg^{2+} in the exchangeable complex in the Lfloor experiment in the Lmag compared with the Lcal treatment, either at depth 0–0.10 m or at depth 0.10–0.20 m, reflected its greater mobility in comparison with Ca^{2+}, as previously reported (Cifu et al., 2004; Juo & Franzluebbers, 2003). The EA and Al^{3+} followed an inverse trend to Mg^{2+}, having decreased more effectively in the Lmag treatment, mainly when the lime were not incorporated into the soil. CEC, in turn, did not differ significantly between Lcal and Lmag treatments, since this variable incorporates the concentration of both bases (Upjohn et al., 2005; Weil & Brady, 2017) and in this soil it is quantitatively more dependent on Ca^{2+} than on Mg^{2+}. Leaf Mg levels also supported the thesis of greater solubility of dolomitic limestone in the soil. The higher concentration of Mg in the leaves was clear in both experiments, when comparing the values in the Lmag and Lcal treatments. In the Lburied experiment, with lower pH values, the application of Lmag was important in increasing leaf Mg concentrations, which were in the control treatment close to the threshold deficiency established by Fernández-Escobar (2017).

Limestone application significantly increased extractable P only in the Lburied experiment, which had a lower initial pH. This effect of soil liming on P bioavailability has also been reported in other studies, especially when using soils with very low initial pH (Holland et al., 2019; Wang et al., 2012). P tends to be less available to plants in very acidic soils, because of the reaction with Fe and Al oxides and their precipitation as $AlPO_4$ and $FePO_4$ (Havlin et al., 2014). However, this increase in the availability of P in the soil had little effect on the increase of P content in the leaves, since only on the last sampling dates of the Lburied experiment did the effect appear consistent, and especially in the Lmag treatment, which had a more pronounced effect on soil pH. Other studies have shown that some trees, including olive, may hold P in the perennial structure (Ferreira, Rodrigues, et al., 2018; Lopes et al., 2021; Rodrigues et al., 2020). Perhaps this buffering effect of the perennial structure, regulating the nutrient concentration in the shoot, associated with the slow action of lime application in the soil, is the reason for this very delayed effect of liming on the P concentration in the olive leaves.

Leaf Mn concentrations in the Lburied experiment, which had the lowest initial soil pH, increased over time in the control treatment. This result may be because of the application of NPK fertilizer. N fertilizers tend to reduce soil pH, because of nitrification of the ammonium form, which generates an excess of H^+ ions during bacterial conversion of NH_4^+ to NO_3^- (Thomas & Hargrove, 2015; Upjohn et al., 2005; Weil & Brady, 2017) and, for this reason, have progressively increased the uptake of Mn by plants. The increase of Mn in the leaves by the application of N fertilizers has also been previously recorded by Lopes et al. (2021) in a soil developed from similar lithological materials. The bioavailability of Mn is also very sensitive to the redox potential of the soil (Sparrow & Uren, 2014). Fertilizer application, by increasing the biological and enzymatic activity of the soil, may have contributed to the consumption of oxygen, reducing the redox potential and temporarily increasing the bioavailability of Mn. In the Lcal and Lmag treatments, the pH increase may have reduced the Mn bioavailability and the Mn levels in the leaves, the results being lower than in the control treatment, as is usually reported (Thomas & Hargrove, 2015; Wakwoya et al., 2022).

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The application of limestone did not significantly influence the soil organic C content, neither the total N nor the C/N ratio. It has sometimes been reported that lime application can reduce soil organic matter by increasing its mineralization, removing an important ecological factor limiting the activity of microorganisms (Havlin et al., 2014; Thomas & Hargrove, 2015). In this study, this did not happen, perhaps because the rise in pH also favoured the growth of weeds and the deposition of organic substrate by the roots of the plants and mycorrhizal fungi, which may have compensated for potential C losses because of the activity of micro-organisms. Weeds can make an important contribution to the deposition of organic substrate in the soil (Silva et al., 2021), as well as mycorrhizal fungi, by channelling plant photosynthates into the soil (Godbold et al., 2006). Although the total organic C did not change with the treatments, the microbial C decreased in the Lfloor experiment, albeit only in the Lmag treatment, and increased in the Lburied experiment with the application of both limestones. Microbial N had the opposite trend to microbial C. The rise in pH leads to greater proliferation of bacteria, while fungi may lose importance (Rousk et al., 2009; Weil & Brady, 2017). In the Lfloor experiment, the pH will have risen enough for the bacteria to dominate the biological activity of the soil, leading to lower levels of microbial C and higher levels of microbial N. In the Lburied experiment, the final pH would have remained quite acidic to limit the presence of bacteria, but the presence of fungi increased, associated with greater availability of organic substrate for decomposition and better conditions for the proliferation of mycorrhizae.

The accumulated olive yield increased with the application of limestone only in the Lburied experiment with a lower initial pH, but showed a tendency towards higher values in the last harvest of the Lfloor experiment with the highest value in the Lmag treatment. Although it is considered that the optimal pH range for the olive tree is not adequately established, it is accepted that the plant vegetates satisfactorily above pH 5.5 (Fernández-Escobar, 2017; Freeman & Carlson, 2005). It has also been observed that the cultivar Cobrançosa produces satisfactorily in soils with much lower pH (Lopes et al., 2020, 2022; Rodrigues et al., 2013; Silva et al., 2021). These results seem to show that in the Lfloor experiment, with an initial pH of 5.5, yield was not much limited by soil acidity. The soil of the Lburied experiment had an initial pH of 4.9, a value low enough for the application of limestone to have increased the accumulated olive yield.

Principal component analysis results stressed that Soil. Ca, Soil.CEC, Soil.pH, Leaf. Mn, Soil. Mg, Soil.P, Leaf. Ca and Yield as some of the most important variables in the Lburied experiment, whereas biological-dependent variables such as Naph.phos, Mic.C, Mic.N and Val.aryl were highlighted in both experiments. Spearman's coefficient values showed that the enzymes Naph.phos and Val.aryl are involved in 9 and 4 correlations, respectively, and Mic.N and Mic.C in 6, with leaf nutrients, chemical soil properties and olive yield. Naph.phos (Naphthol-AS-BI-phosphohydrolase) is an enzyme associated with the P cycle in soil that normally accompanies alkaline phosphatase and acid phosphatase activities (Sampaio et al., 2017). It is therefore clear that the limestone treatments influenced the dynamics of P in the soil even though the variables measured in the plant, especially leaf P, were less sensitive to the treatments because of the buffering effect of the perennial structure on the concentration of the nutrient in the leaves as above mentioned. The enzyme arylamidase catalyses the hydrolysis of an N-terminal amino acid from peptides, amides or arylamides in soils (Acosta-Martínez & Tabatabai, 2001) and it has been suggested that this enzyme plays a major role as the initial reaction-limiting step in mineralization of organic N in soils (Dodor & Tabatabai, 2007). Its high activity in this study, and its relationship with several soil properties, is indicative of the transformations that occurred in soil organic matter and identified by the Mic.N and Mic.C variables, although these tended to vary differently with soil treatments.

5 | CONCLUSIONS

Olive yield increased consistently with the application of limestone in the Lburied experiment in which the initial pH was 4.9. In the Lfloor experiment, with an initial pH of 5.5, there were no significant differences in accumulated olive yield between the limestone treatments and the control, although in the last harvest the control yielded less than the Lmag treatment. Dolomitic limestone was more effective in pH increase than calcitic limestone, insofar as in the soil depth of 0.10–0.20 m only in the Lmag treatments of both experiments the values were significantly higher than in the controls. The application of dolomitic limestone seems to be justified in olive groves under no-tillage systems, where limestone should not be incorporated into the soil to avoid damage to the root system and not to favour soil erosion. Lime application increased the accumulated olive yield only in the Lburied experiment because of the very low initial soil pH (4.9). In the Lfloor experiment, with an initial soil pH of 5.5, there were no significant differences between treatments, also because of the good adaptation of 'Cobrançosa' to acid soils.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Margarida Arrobas https://orcid.org/0000-0002-4652-485X
Nuno Conceição https://orcid.org/0000-0001-6954-8144
Ermelinda Pereira https://orcid.org/0000-0002-9431-5059
Sandra Martins https://orcid.org/0000-0002-7519-4001
Soraia Raimundo https://orcid.org/0000-0002-6505-1445
Cátia Brito https://orcid.org/0000-0003-2370-6084
Carlos Manuel Correia https://orcid.org/0000-0003-2482-7873
Manuel Ângelo Rodrigues https://orcid.org/0000-0002-5367-1129

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