

Article

Liming and Phosphate Application Influence Soil Carbon and Nitrogen Mineralization Differently in Response to Temperature Regimes in Allophanic Andosols

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Abstract: Andosols are characterized by high organic matter content and play a significant role in carbon storage. However, they have low phosphorus fertility because of the high phosphate-fixing capacity of active aluminum. For agricultural use of Andosols, it is necessary to ameliorate its poor phosphorus fertility by applying lime and high doses of phosphate fertilizers. The objective of the present study was to clarify how such soil amendments affect the mineralization of soil organic carbon (C) and nitrogen (N) in allophanic Andosols under different temperature regimes. The soil was treated using combinations of liming and heavy phosphate application, followed by incubation under different temperature conditions. The N mineralization and the soil CO₂ evolution rate were measured periodically. The patterns of N mineralization were analyzed by fitting them to first-order kinetics. Liming increased C and N mineralization irrespective of temperature, and the increase was further enhanced by phosphate application. Kinetic analysis of the N mineralization curve indicated lowering of the activation energy of N mineralization reactions with phosphate application, suggesting that P application may accelerate N mineralization at lower temperatures. These findings provide a basis for developing soil management strategies to reduce the loss of soil organic matter.

Keywords: Andosols; soil carbon; global warming; phosphorus; phosphate; liming; nitrogen; mineralization; temperature



Citation: Matsuoka-Uno, C.; Uno, T.; Tajima, R.; Ito, T.; Saito, M. Liming and Phosphate Application Influence Soil Carbon and Nitrogen Mineralization Differently in Response to Temperature Regimes in Allophanic Andosols. *Agriculture* **2022**, *12*, 142. <https://doi.org/10.3390/agriculture12020142>

Academic Editor: Laura Zavattaro

Received: 9 December 2021

Accepted: 20 January 2022

Published: 21 January 2022

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1. Introduction

A huge amount of carbon (C), 1500 Pg C, approximately twice the amount present in the atmosphere and thrice the amount in terrestrial plant biomass, is stored in the soil as organic matter [1]. Furthermore, any variation in the soil organic matter content significantly impacts the global carbon cycle. In arable soils, the amount of soil C is strongly influenced by environmental factors such as temperature and agricultural management practices such as tillage. Life-cycle inventory analysis of greenhouse gases (GHGs) in various crop production systems in northern Japan revealed that carbon dioxide (CO₂) emissions from soil were the largest source of GHG emissions [2]. Various reports have warned that the increase in decomposition of soil organic matter due to global warming will further accelerate the atmospheric CO₂ concentration [3–5]. In order to take countermeasures against global warming, it is essential to predict the potential changes in soil C status in response to environmental changes and human activities (such as agricultural soil management practices).

Andosols are of volcanic origin and are one of the most widely distributed soils in Japan (31% of the country's land area and 47% of upland arable fields) [6]. Andosols are characterized by high organic matter content, resulting in high soil C storage capacity and

excellent physical and water retention properties. However, they have low phosphorus fertility because of the high phosphate-fixing capacity of active aluminum. For agricultural use of Andosols in Japan, when uncultivated Andosols are converted into arable lands, it is recommended to ameliorate the soil by applying a mixture of phosphate fertilizers (e.g., fused phosphate and superphosphate) equivalent to 10% of the phosphate absorption coefficient (PAC) of the soil [7]. Such large amounts of phosphate fertilization not only increase the amount of available phosphate in the soil, but also increase phosphorus fertility of the soil by masking some of the phosphate fixing sites of clay particles and decreasing the phosphorus adsorption capacity [6,8]. These amendments may increase the activity of soil microorganisms that were previously limited by the phosphorous deficiency and acidity of the soil and may promote the decomposition of soil organic matter. Munevar and Wollum [9] reported that the addition of phosphate and N increased the mineralization of soil organic C and N in the Andosols of Colombia. Ogasawara et al. [10] reported that soil N mineralization increased with the addition of a mixture of fused phosphate and superphosphate in the Andosols of northern Japan. These incubation experiments were conducted at about 30 °C. However, in the northern part of Japan under cool-temperate climate, the average annual soil temperature is 10–15 °C and rarely exceeds 25 °C, even in summer. Although a large number of studies on temperature dependency of soil organic matter decomposition have been reported, e.g., [3–5], the effect of soil amendments on soil organic matter decomposition focusing on temperature regime has rarely been reported.

The objective of the present study was to clarify how these soil amendments affect mineralization of soil organic C and N, with an emphasis on temperature response. We hypothesize that soil available phosphate limits C and N mineralization in an allophanic Andosol and that this effect is more evident in lower temperature regime. In the present study, we used a soil sample from an uncultivated land with an allophanic Andosols under cool-temperate climate (annual mean temperature 10.6 °C) and treated the soil with different combinations of lime and phosphate. Phosphate was applied at the rate of 5% and 20% of PAC, as indicated in the study by Yamamoto and Miyasato [7]. Mineralization of soil organic carbon and nitrogen was examined by incubating the soil under different temperature regimes.

2. Materials and Methods

2.1. Soil

The soil was collected from the surface layer (0–20 cm) of an uncultivated land (39°44'56.4" N, 141°08'16.8" E) under *Pinus densiflora* trees in Morioka City, Iwate Prefecture, Japan, after removing the litter layer of soil. The physicochemical properties of the soil were as follows: texture, LiC; total C, 186 g C kg⁻¹ dry soil; total N, 11.7 g N kg⁻¹ dry soil; ammonium N, 63 mg N kg⁻¹ dry soil; nitrate N, 65 mg N kg⁻¹ dry soil; pH (H₂O), 5.3; PAC (phosphate absorption coefficient), 23.3 g P₂O₅ kg⁻¹ dry soil; available phosphate, 0.016 g P₂O₅ kg⁻¹ dry soil (Truog method); maximum water holding capacity, 0.80 cm³/cm³. Analytical methods of soil are described in the Supplementary Materials. The soil was classified as an allophanic cumulic Andosol according to the Soil Classification System of Japan [6].

2.2. Design for Incubation Experiment

Fresh, moist soil samples that were air-dried, sieved through a 2 mm mesh, and treated with different levels of phosphate and lime were used for the incubation experiment. In the non-liming treatment, two levels of potassium phosphate monobasic (0% for control, 5% and 20% of PAC; equivalent to about 11.6 Mg P₂O₅ ha⁻¹, 46.6 MgP₂O₅ ha⁻¹) were added to the soil samples. In the liming treatment, calcium hydroxide (5.5 g kg⁻¹ dry soil) was used to adjust the soil pH (to ~6.0) before adding potassium phosphate at the rate of 0%, 5%, and 20% of PAC. Soil sample was incubated for each treatment in triplicate (see the Supplementary Materials). The incubation temperatures were 20 and 30 °C. An additional temperature level, 25 °C, was set for N mineralization in the non-liming treatment, because

preliminary experiment showed another temperature level may be required for more reliable kinetic parameters of N mineralization (Section 2.4) in the non-liming treatment.

The treated soil samples were put in separate 100 mL polyethylene vials, individually covered with aluminum foil, and incubated for 120 days. Throughout the incubation period, the soil moisture condition was maintained at 50% of the maximum water holding capacity of the soil. The pH (H₂O) and available phosphate content (Truog method) of the soil samples were measured periodically throughout the incubation period.

2.3. C Mineralization: CO₂ Evolution

Organic C mineralization was estimated by periodically measuring the rate of CO₂ evolution from the soil (Figure S1). The aluminum foil was removed from the incubated samples, and the polyethylene vials were individually placed in 485 mL wide-mouthed glass bottles with a septum for gas sampling. The bottles were incubated at the respective incubation temperatures. About 1 h and 24 h after the bottles were incubated, 1 mL gas was collected from the bottle using a gas-tight syringe, and the CO₂ concentration was measured using a gas chromatograph (GC-8A, Shimadzu, Kyoto, Japan) equipped with a thermal conductivity detector and a Shincarbon ST column (Shinwa Chemical Industries Ltd., Kyoto, Japan). The CO₂ evolution rate was calculated from the increase in the CO₂ concentration during this period. The soils in the polyethylene vials after CO₂ assay were back to the incubator for further CO₂ evolution measurement.

2.4. N Mineralization

After the designated incubation period, mineral N was extracted from the soil samples using 2 N KCl. The nitrate-N, nitrite-N, and ammonium-N contents of each extract were estimated using an autoanalyzer (QuAatro 2-HR, BL-TECH Co., Tokyo, Japan), and the sum of these N values was considered as the mineral N content of the respective sample.

2.5. Kinetic Analysis of N Mineralization Curve

The mineral N contents obtained in this study were presented in the form of a N mineralization curve, which was analyzed using the method proposed by Sugihara et al. [11]. This method assumes that the N mineralization curve corresponds to an equation of the first-order reaction (Equation (1)), and the relationship between the mineralization rate constant and temperature follows the Arrhenius law (Equation (2)).

$$N_t = N_0 \{1 - \exp(-k \cdot t)\} \quad (1)$$

$$k = A \cdot \exp(-E_a/R \cdot T) \quad (2)$$

where N_t and N_0 , amount of mineralized N at time t and infinite time, respectively (mg N kg⁻¹ soil); k , mineralization rate constant (day⁻¹); E_a , activation energy (kJ mol⁻¹); T , absolute temperature (K); R , universal gas constant (8.31 J mol⁻¹ K⁻¹); A , Arrhenius constant.

Curve fitting of the data was performed using the Solver program of Microsoft Excel 2019 (Microsoft Corporation, Redmond, WA, USA). Fitness was evaluated on the basis of the minimum Akaike information criterion values. Parameters obtained by this non-linear curve fitting were compared among the treatments by Bonfferoni method (see the Supplementary Materials).

2.6. Statistical Analyses

Statistical analyses were conducted using the JMP software (SAS Institute Japan, Tokyo, Japan) and the data analyses tools of Microsoft Excel 2019.

3. Results

3.1. Change of Available Phosphate and pH of Soil during Incubation

The changes in pH are shown in Figure 1. The pH of soil samples subjected to liming treatment was initially adjusted to ≈6.0. In all treatments, the pH gradually decreased,

probably due to the accumulation of nitrate N, while a small increase in pH during the early incubation period was found in some treatments. Overall, the change in pH during the incubation period was within one unit.

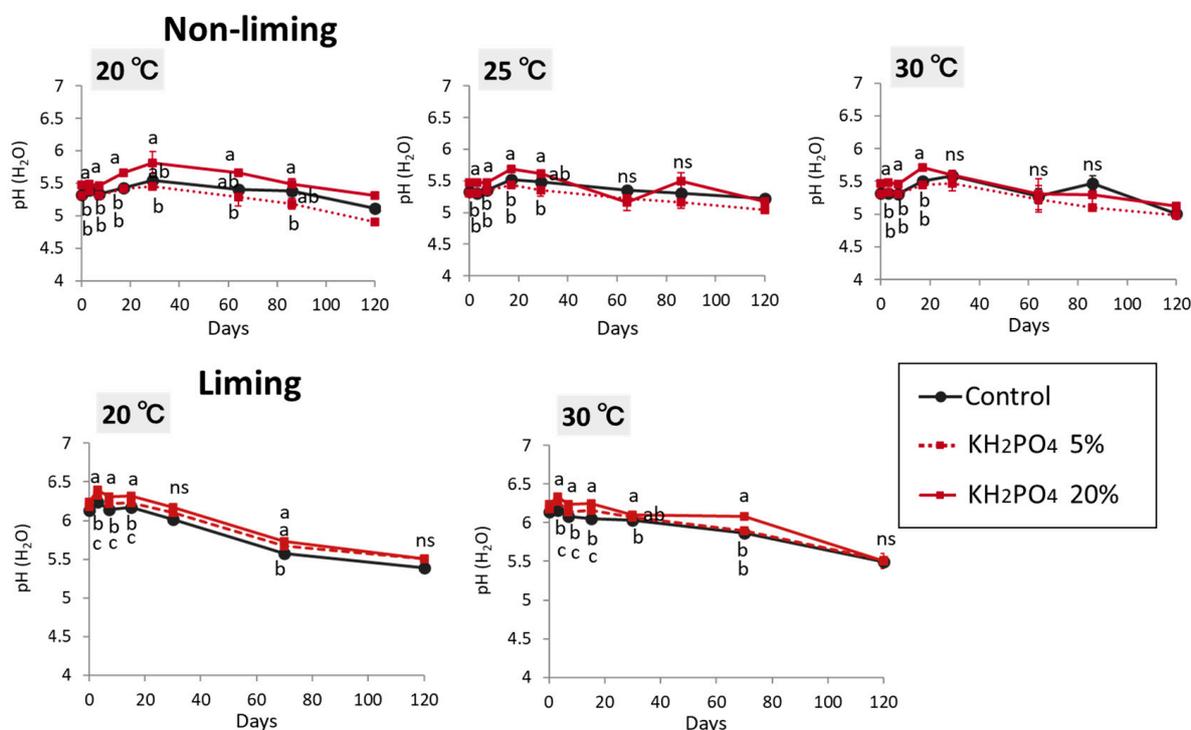


Figure 1. Change of soil pH (H₂O) during the incubation period. Vertical bars indicate the standard error of means ($n = 3$). Different letters indicate significantly different on the same day (Tukey's multiple comparison test, ns; not significantly different). The data on day 120 in non-liming treatment were not replicated, and therefore the statistical analyses were not performed.

The change in the available phosphate at 20 °C is shown in Figure 2. The trend at 30 °C was almost the same as that observed at 20 °C (data not shown). Available phosphate decreased immediately after the addition of potassium phosphate due to the quick fixation of phosphate, and then remained constant, for both application levels. In the liming treatment, the amount of available phosphate was higher than that in the non-liming treatment. It may have been due to a somewhat higher pH in the liming treatment.

3.2. CO₂ Evolution

The change in the rate of CO₂ evolution from the soil during the incubation period is shown in Figure 3. The pattern of changes in the CO₂ evolution rate differed between the non-liming and liming treatments. In the non-liming treatment, the CO₂ evolution rate increased with time, peaked at 40–50 days, and then decreased. However, liming treatment increased the overall CO₂ evolution rate of the soil, compared to the non-liming treatment. There was a peak in CO₂ evolution at the beginning of the incubation period, followed by a decline and a small peak at ≈40 days, similar to that in the non-liming treatment. The total CO₂ evolution during the incubation period (Figure 4) was estimated by integrating the curves of the CO₂ evolution rate up to 84 days. In the non-liming treatment, phosphate application was not significant in terms of the total amount of CO₂ evolved. In contrast, in the liming treatment, phosphate application increased total CO₂ evolution at 30 °C (Figure 4).

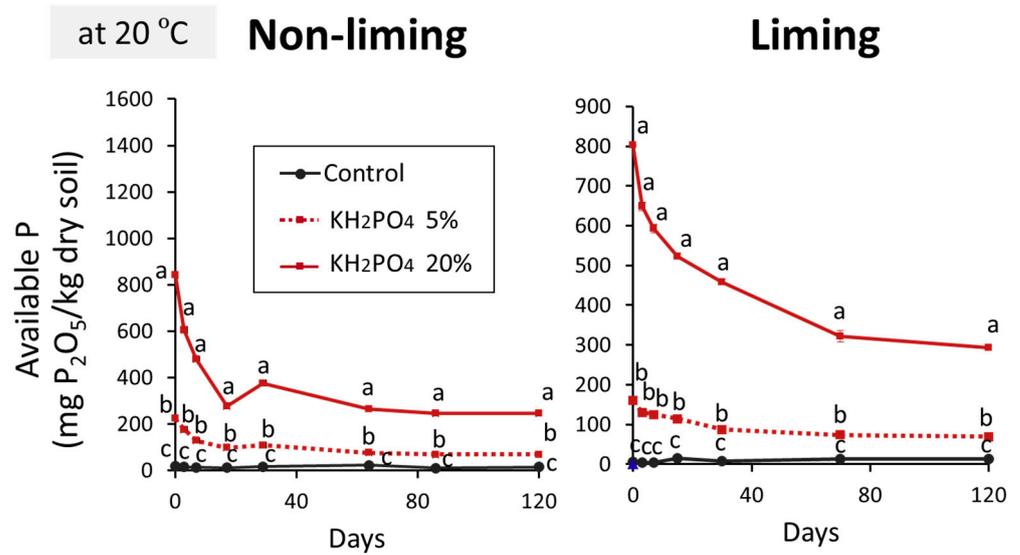


Figure 2. Change of available phosphate in soil (Truog method) at 20 °C. Vertical bars indicate the standard error of means ($n = 3$). Different letters indicate significantly different on the same day (Tukey’s multiple comparison test).

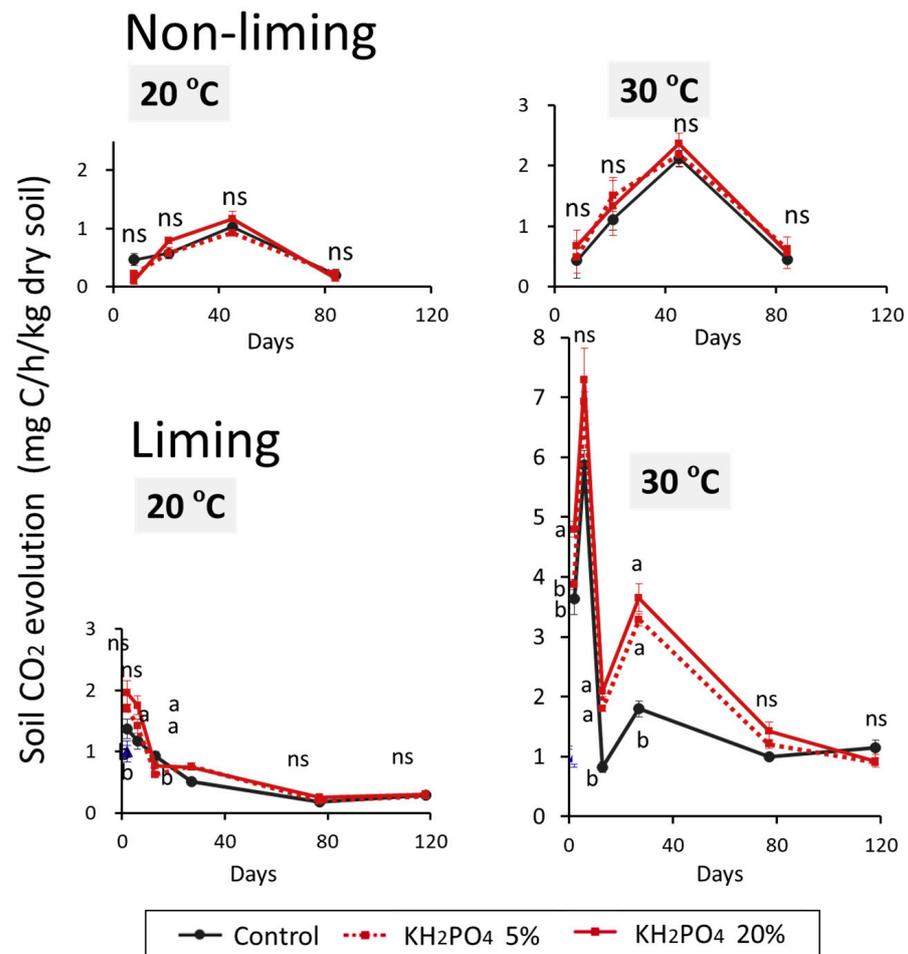


Figure 3. CO₂ evolution rates at different temperatures. Vertical bars indicate the standard error of means ($n = 3$). Different letters indicate significantly different at each temperature (Tukey’s multiple comparison test; ns, not significantly different).

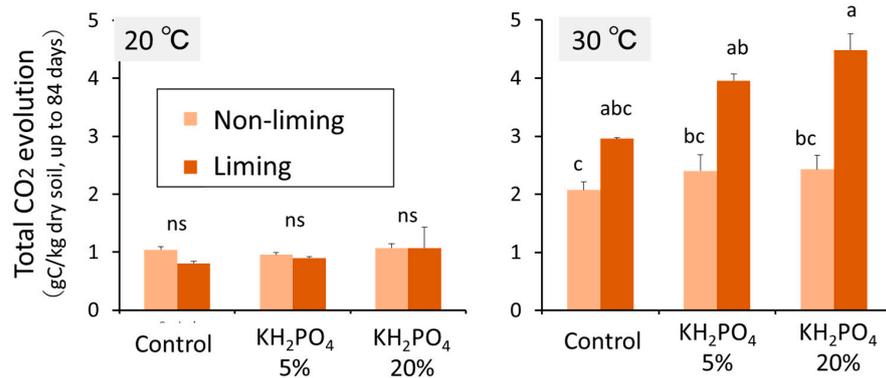


Figure 4. Total C mineralization estimated up to 84 days by CO₂ evolution rate at different temperatures. Vertical bars indicate the standard error of means (*n* = 3). Different letters indicate significantly different at each temperature (Tukey’s multiple comparison test; ns, not significantly different). Two ways ANOVA (temperature × liming) showed both temperature and liming treatments showed *p* < 0.01. Since the CO₂ evolution data at 20 °C were obtained only up to day 84 (Figure 3), total CO₂ evolution at 30 °C was also estimated up to day 84. The data for day 84 in the liming treatment was extrapolated from the data for day 77 and day 120.

3.3. N Mineralization Curves and Their Kinetic Analysis

The accumulation of mineral N in the soil during the incubation period is shown in Figure 5. In the non-liming treatment, N mineralization at 20 °C increased with the application of phosphate at the rate of 20% PAC. In the liming treatment, N mineralization increased to some degree for both levels of phosphate application. The N mineralization parameters were obtained by fitting the N mineralization curve to the first-order reaction (Table 1). The mineralization rate constants were not significantly affected by both phosphate application and liming. The activation energy (*E_a*) in the non-liming treatment significantly decreased with phosphate application (20% of PAC). Liming increased the amount of potentially mineralizable N (*N₀*).

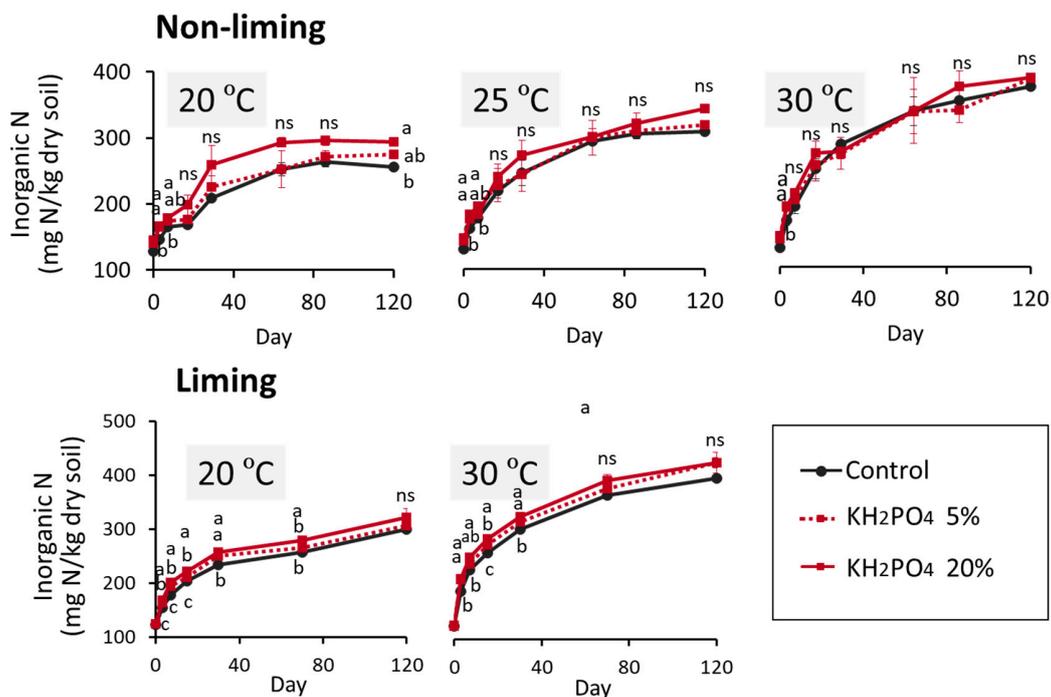


Figure 5. Change of mineral N in soils at different incubation treatments and temperatures. Vertical bars indicate the standard error of means (*n* = 3). Different letters indicate significant difference at each temperature (Tukey’s multiple comparison test; ns, not significantly different).

Table 1. Nitrogen mineralization parameters of soil incubated at different treatments and temperatures.

Treatment		No *		k *		Ea *	
Liming	Phosphate	mg N/kg Dry Soil		/day, 25 °C		kJ/mol	
			#		#		#
Non-liming	Control	200		0.022		5.39	a
	KH ₂ PO ₄ 5%	178	ns	0.021	ns	5.03	a
	KH ₂ PO ₄ 20%	188		0.029		3.45	b
	(average)	(189)		(0.024)		(4.62)	
Liming	Control	228		0.018		5.92	
	KH ₂ PO ₄ 5%	240	ns	0.016	ns	6.13	ns
	KH ₂ PO ₄ 20%	237		0.019		6.06	
	(average)	(235)		(0.018)		(6.04)	
	##	*		ns		ns	

* No, potentially mineralizable N; k, mineralization rate constant; Ea, activation energy. # Different letters (a, b) indicate statistical significance among phosphate addition treatments by the Bonferroni method ($p < 0.05$, ns: not significant). ## Statistical difference between the non-liming and liming treatments (*: $p < 0.05$, ns: not significant).

4. Discussion

In the present study, we examined the effects of liming and heavy phosphate application on C and N (N) mineralization in soil samples from the uncultivated allophanic Andosols of Japan.

The effect of liming showed the same trend irrespective of the temperature. Liming increased the rate of CO₂ evolution during the early stages of incubation (Figure 3). The N mineralization curve also showed the same trend—liming increased N mineralization in the initial stages of incubation. This result was supported by the kinetic analysis of the N mineralization curve, which showed that N mineralization potential, N₀, was increased by liming (Table 1).

In contrast, the effect of phosphate application varied with liming and incubation temperature. The total CO₂ evolution, which is the integrated value of the CO₂ evolution during the incubation period (Figure 4), increased with phosphate application and was more pronounced at 30 °C in the liming treatments, whereas it was not as evident in the non-liming treatments. N mineralization in the non-liming treatment at 20 °C was promoted by phosphate application. N mineralization parameters based on the kinetic analysis of N mineralization curves showed that the activation energy, Ea, in the non-liming treatment decreased with an increase in the phosphate application rate (Table 1).

The enhancement of soil organic matter decomposition by liming is well documented [12–16]. This reaction occurs partly because of the increase in microbial activity due to the neutralization of soil acidity, and partly because of the solubilization of organic matter. Liming releases organic matter trapped in soil aggregates by the dispersion of clay. Takahashi et al. [17] reported that the addition of lime to Andosols broke down a part of the Al–humus complex and led highly humified soil organic matter to more decomposable. Another source of solubilized organic matter might be microbial biomass. Marumoto et al. [18] reported that lime application killed some microbial biomass (especially fungi) and caused a rapid growth of microorganisms that utilize the dead microbial biomass. The microorganisms responsible for mineralization immediately after liming—as observed in the early stages of incubation in the present study—are probably the newly proliferated microorganisms, “fast growers” that utilize the solubilized and easily degradable fraction of organic matter. These “fast growers” may be more active at a higher temperature (30 °C), and more phosphate may be required for their activity (Figure 4).

The relationship between the activation energy (Ea) of N mineralization and the average available soil phosphate during the incubation period (Figure 6) shows that the increase in available phosphate leads to a decrease in Ea in the non-liming treatment, indicating that the mineralization reaction occurs more readily at lower temperatures. In

terms of temperature dependency of organic matter decomposition, recalcitrant organic matter may show higher activation energy and thus may show its higher temperature dependency than labile organic matter [19]. In contrast to liming as discussed above, it is unlikely that the addition of phosphate may have had a significant impact on organic matter quality. In the non-liming treatments, the microorganisms involved in C and N mineralization may be “slow growers” that decompose indigenous soil organic matter. Their activity is probably slower than that of the “fast growers” observed during the early stages of liming treatments. The microorganisms might have adapted to temperatures lower than 30 °C, and their activity in this temperature range might have been enhanced by phosphate addition.

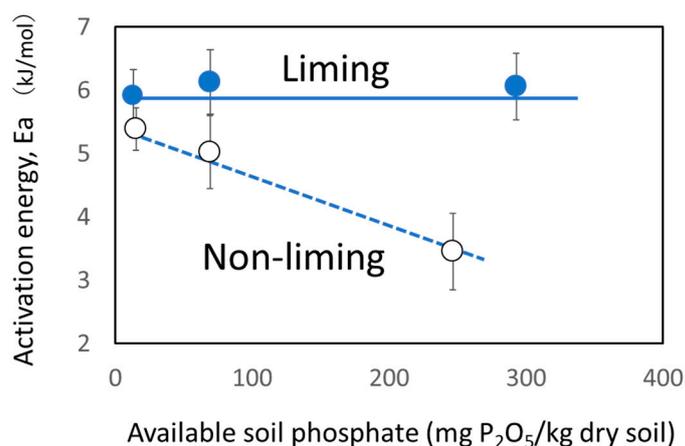


Figure 6. Relationship between apparent activation energy of nitrogen mineralization and available soil phosphate. The available soil phosphate (Trouw method) indicated here is the average of the available soil phosphate values during the incubation period (15–120 days, 20 °C). Bars indicate the standard error of means ($n = 3$). Lines in figure were arbitrarily drawn.

Several studies have reported that soil C and N mineralization is limited by the phosphate availability in highly weathered tropical soils with high phosphate-fixing capacity [20,21] and in cool, temperate soils rich in organic content, such as Andosols [9,22–24]. These studies did not refer to temperature dependency of the mineralization. Saito [25] examined the N mineralization characteristics of various soils in the northern Tohoku region of Japan and found a negative correlation between available p and E_a . This corresponds with the present result in non-liming treatment (Figure 6). Microbial populations at lower temperatures than the optimum require higher concentrations of substrates due to lowering affinity to substrate [26]. In the control soil in the non-liming treatment, available phosphate was kept very low (Figure 2), and therefore that phosphate may be deficit for microorganisms. The addition of phosphate may mitigate such phosphate deficit for soil microorganism and may result in lowering E_a . Microbial community analysis of a wide range of Japanese Andosols showed that fungal diversity decreased with increasing soil available phosphate, and *Mortierella* tended to be one of the dominant fungal groups [27]. This also might be related to changes in the temperature responsiveness of N mineralization to phosphate application, as observed in E_a (Figure 6).

Global warming is threatening to accelerate the loss of soil organic carbon. In fact, the thawing of frozen soils in polar regions is becoming a reality [28,29]. This may be also a serious issue in cool and cold temperate regions, where soils are rich in organic matter. In Japan, higher doses of phosphate fertilizers are used in agricultural production, partly because Andosols, which have a high phosphate-fixing capacity, are widely distributed in the arable lands. This practice has increased the available phosphate in arable soils [30]. Intensive fertilization of arable soils may accelerate the loss of soil organic carbon.

For sustainable agricultural production in the changing climatic conditions, soil management practices must be improved to facilitate soil carbon storage [31]. Many studies have

been conducted for this purpose [32,33]. The present study indicates that the effect of intensive fertilizer management on organic matter decomposition should be investigated more systematically and extensively, as well as being reflected in sustainable soil management.

5. Conclusions

Conversion of uncultivated Andosols to arable lands generally requires liming treatments and heavy phosphate applications to improve crop production [6,8]. The effects of liming and heavy application of phosphate on soil C and N mineralization in allophanic Andosols were investigated under different temperature regimes. Liming led highly humified organic matter to more decomposable [16,17,24] and increased the easily degradable fraction of organic matter, and as a result, the microbial communities that proliferated using these fractions became more active with the application of high amounts of phosphate. In contrast, in the non-liming treatment, phosphate increased soil N mineralization at lower temperatures. These findings support our hypothesis raised in the Introduction section and also provide a basis for the development of a model for predicting soil C and N mineralization. The model may contribute to the development of management strategies to reduce the loss of soil organic matter in the face of global warming.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agriculture12020142/s1>, Analytical methods of soil, Incubation of soil, Curve fitting of N mineralization curve. Figure S1: Measurement of soil CO₂ evolution rate.

Author Contributions: Conceptualization, M.S.; methodology, C.M.-U., T.U., R.T., T.I. and M.S.; data analysis, C.M.-U., R.T. and M.S.; writing, C.M.-U. and M.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was in-part supported by ESPEC Foundation for Global Environment Research and Technology (ESPEC Prize for the Encouragement of Environmental Studies).

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We are grateful to Shintaro Hara for his technical support of gas chromatographic analysis.

Conflicts of Interest: The authors declare no conflict of interest.

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