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Effect of soil acidification on regrowth of orchardgrass (*Dactylis glomerata*) under application of grazing cattle dung, cattle manure compost, and chemical fertilizer

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Abstract

Orchardgrass (*Dactylis glomerata*) persists poorly in acidic soils. Not many studies have looked into the effects of fertilizers in improving orchardgrass persistence within acidic soils. We conducted experiments on 64 individual potted orchardgrass plants, which were defoliated to 5 cm and assigned to one of the following four treatment groups: unfertilized control (CNT), chemical fertilizer (CHE), grazing cattle dung (DNG), and cattle manure compost (CMP). Half the pots in each treatment received aluminum sulfate solution to induce further soil acidification (Al-add), while the others received water (no-Al). On days 20 and 47, after defoliation, soil properties and dry weights of aboveground biomass (AGB) (separated into leaves and stubble) and roots of four pots in each treatment group were measured. Al-add induced soil acidification in all fertilizers across the experiment ($p<0.05$). On day 20, AGB and leaves in CHE was increased by acidification ($p<0.05$), which was not observed in other fertilizer treatments ($p>0.1$). Stubble growth increased following acidification in all fertilizer treatments ($p<0.05$). Acidification did not increase AGB on day 47; no effect was seen on root growth at either day 20 or 47 ($p>0.1$). On day 20, soil concentrations of inorganic nitrogen (IN), nitrate-nitrogen, and ammonium-nitrogen (NH_4-N) were significantly elevated in Al-add pots ($p<0.05$). The increment was greater in CHE and CMP than in CNT and DNG on day 20, with a similar trend being observed for IN and NH_4-N concentrations at day 47. IN and NH_4-N concentrations in DNG with no-Al increased over the regrowth period. These results indicate that orchardgrass regrowth in acidic soils can be improved by fertilizer addition, depending on fertilizer type. The increased concentration of soil IN, induced by soil acidification, is likely to be one of the factors encouraging growth. This increase of regrowth may favor the persistence of orchardgrass in strongly acidic soils.

KEYWORDS

acidic soil, forage regrowth, inorganic nitrogen, pasture management, soil nutrient

1 | INTRODUCTION

Orchardgrass (*Dactylis glomerata* L.) is a widely used temperate forage grass. In pasture-based farming, the persistence of forage grass is one of the most important factors for sustainable production (Tozer et al., 2011). However, this grass species often declines within a couple of years of planting (Scott et al., 2000), with particularly low persistence under acidic soil conditions (Hojito et al., 1987; Poozesh et al., 2010).

Acidic soil affects plant growth by altering the availability of soil nutrients and releasing aluminum ions harmful to plants. It induces the accumulation of inorganic nitrogen (Aciego Pietri & Brookes, 2008; Kemmitt et al., 2005) and limits the availability of phosphorus and exchangeable cations to plants (Follett & Wilkinson, 1985; Hojito et al., 1987; Naidu et al., 1990). The characteristics of soil nutrients in pastures also differ under different pasture management regimes, such as hay cutting and livestock grazing (Bryan et al., 2019; Franzluebbers & Stuedemann, 2010), which can have a compounded impact on the effect of acidic soil on pasture plants' growth.

We have previously shown that the dominance response of orchardgrass to soil acidity was different between hay cutting and cattle grazing. Orchardgrass sown in hay meadows survived better than other plant species and became the dominant species more frequently under strongly acidic soil conditions compared to mildly acidic soil. We did not observe any significant effect of soil acidity under cattle grazing conditions (Kakihara & Ogura, 2021). This may be due to the difference in pasture management regimes, such as fertilizer application. In general, chemical fertilizer and manure compost is used in hay meadows, whereas pastures utilize dung and urine which are directly deposited onto the field by animals. Chemical fertilizers, compost, and cattle manure affect the properties of pasture soils, which in turn changes the growth and species composition of plants in the pasture, even though these fertilizers may contain the same amount of nutrients (Min et al., 2003; Mauchamp et al., 2016).

The effect of fertilizer type on the regrowth of orchardgrass, when grown in hay meadows, needs to be studied to determine its mechanism of persistence in strongly acidic soil. Therefore, in the present study, we investigated the effect of fertilizer type on the concentration of soil nutrients and the regrowth of orchardgrass under different soil acidification levels by performing a pot experiment.

2 | MATERIALS AND METHODS

2.1 | Study site and pot experiment

The study was conducted at the Kawatabi Field Science Center (FSC), Graduate School of Agricultural Science, Tohoku University (Osaki, Miyagi, Japan; 38.75°N, 140.76°E, 200–250 m above sea level). The mean annual air temperature at the study site in 2019 was 11.1°C, with a maximum temperature of 35.8°C in August and a minimum temperature of –7.8°C in January (Japan Meteorological Agency, 2020).

To obtain individual orchardgrass plants, seeds (cv. Akimidori II) were sown in a rectangular plastic planter on November 2, 2018, and 64 seedlings were transplanted into 64 pots (*i.e.*, one plant per plastic pot with ⌀12.5 cm and 15 cm depth) filled with sieved soil (<6 mm) on June 10, 2019. The potting soil was obtained from a hay meadow in the FSC. The soil type was classified as Haplic non-allophanic Andosol according to the Soil Classification System of Japan (The fifth committee for soil classification and nomenclature, 2017), Pachic Melanudand according to Soil Taxonomy (Soil Survey Staff, 2014), or Aluandic Andosol in The World Reference Base classification (IUSS Working Group WRB, 2015).

The chemical composition of the potting soil is described in Table 1. The orchardgrass plants were grown outdoors for three months, then defoliated at the height of 5 cm on September 12, 2019, to impose the same cutting condition on all individuals. After defoliation, the pots were divided into four fertilizer treatments groups: 1) unfertilized control (CNT), 2) chemical fertilizer of N, P_2O_5, K_2O at 34, 28, and 28 kg/ha as urea, double superphosphate, and potassium chloride, respectively (CHE), 3) 10 g of grazing cattle dung (DNG), and 4) 2.39 g of cattle manure compost (CMP) (Table 1). The grazing cattle dung was collected from pastures dominated by orchardgrass, tall fescue (*Festuca arundinacea*), redtop (*Agrostis alba*), and sweet vernalgrass (*Anthoxanthum odoratum*), grazed by Japanese Black cows. For one month, the cattle manure compost was produced by mixing cattle manure and sawdust in a machine, 1–2 times a week. The dung and compost were kept refrigerated at 8°C for four days until application. The amount applied was determined based on the nutrient content in 10 g of cattle dung, with total nitrogen (TN) for compost and TN, phosphorus, and potassium for chemical fertilizer. The amount of applied cattle dung was equivalent to that of 150 days of grazing by 3.3 cattle/ha, which is the conventional grazing management in the FCS. To half of the pots in each fertilizer treatment (8 pots per treatment), 1 g of aluminum sulfate 14–18 hydrate ($Al_2(SO_4)_3 \cdot 14–18H_2O$) was added per pot as a water solution to induce further soil acidification (Al-add), whereas the other half received water only (no-Al). The fertilizers and aluminum sulfate were top-dressed immediately after the defoliation. The pots were placed outdoor and irrigated every 2–3 days to maintain soil moisture throughout the experiment. Excess water was drained out of the pots.

2.2 | Measurement

The measurements and samplings were carried out 20 and 47 days after the defoliation. The number of tillers was recorded, then aboveground biomass (AGB) was harvested at 0 cm height from the soil. For each pot, the AGB was divided into leaf samples (≥ 5 cm from the soil) and stubble samples (0–5 cm from the soil). Both the leaf and stubble samples were force air-dried for 48 h at 70°C, and then their dry weights were obtained. Roots were collected by gentle washing in water, then air-dried and weighed using the same protocol as for AGB.

Two types of soil samples were collected from each pot: soil at a depth of 0–5 cm (100 g) was used to measure soil pH and exchangeable acidity (y_1), and that at a depth of 0–15 cm (500 g) was used to analyze the concentration of soil nutrients. These soil samples were dried at room temperature, sieved (using a 2 mm mesh) to remove gravel, then stored for the subsequent analysis. Soil pH was measured using 15 g of dried soil by dipping the glass electrode of a pH meter into a 1:2.5 soil to water suspension. The y_1 value was measured using 10 g of dried soil by titrating a 1 M potassium chloride (KCl) extract (soil:solution = 1:2.5) with 0.1 M sodium hydroxide (NaOH) to the endpoint of phenolphthalein (Mori & Shimada, 1970). The value of y_1 is closely related to the amount of KCl extractable Al (Saigusa et al., 1980), so y_1 was used as an indicator of exchangeable Al. TN and total carbon (TC) were measured by dry combustion. Nitrate-nitrogen (NO_3-N) and ammonium-nitrogen (NH_4-N) were evaluated by the hydrazine sulfate reduction and indo-phenol methods, respectively.

Inorganic nitrogen (IN) was estimated as total amount of NO_3-N and NH_4-N. Available phosphorus (P_2O_5) was determined by the Bray-2 method. Potassium oxide (K_2O), calcium oxide (CaO), and magnesium oxide (MgO) were quantified via atomic absorption spectrometry after Schollenberger extraction.

2.3 | Data analysis

Before performing data analysis, one pot data in CMP with no-Al was excluded as it showed extremely sparse roots due to a large number of beetle larvae in it. No larvae or traces were observed in all the other pots.

Dry matter yield per tiller was considered to be the amount of regrowth of orchardgrass as the number of tillers per pot differed among the pots (4–15 tillers per pot), which might influence the total dry matter yield per pot.

Two-way analysis of variance (ANOVA) for each sampling day (days 20 and 47) was performed to examine the effect of fertilizer and acidification treatment on the soil acidity, dry matter weights of the AGB, leaves, stubble, and roots of a plant. If significant interactions were detected, post hoc analysis for each treatment was performed by pairwise multiple comparisons with Holm's method. The soil pH, y_1, and weight of each part of the plant were used as response variables, and the fertilizer and acidification treatment were used as fixed effects. Thus, the model is given by:

$$Y = \beta_0 + \beta_1 \text{ Fertilizer} + \beta_2 \text{ Acidification} + \beta_3 \text{ Interaction}$$

where Y is either soil pH, y_1, or weights of each part of the plant. In the equation of the models, 'Fertilizer', 'Acidification', and 'Interaction' represent the fertilizer treatment, acidification treatment, and the interaction between 'Fertilizer' and 'Acidification', respectively. β_0 is the intercept, β_1, β_2, and β_3 represent coefficients of the exploratory variables and their interaction.

All analyses were performed at a significance level of 0.05 using the ‘lm’, ‘anova’, and ‘interaction’ functions of the ‘base’ package, ‘emmeans’ functions of the ‘emmeans’ package (version 1.4.5), and the ‘glht’ function of the ‘multcomp’ package (version 1.4.12) in R version 3.6.1 (R Core Team, 2019).

3 | RESULTS

3.1 | Soil pH and exchangeable acidity

Soil acidification was induced significantly by the addition of aluminum sulfate in all fertilizer treatments ($p < 0.05$, Table 2). The pH values were lower in Al-add (5.07–5.16) than in no-Al (5.61–5.63) on day 20. A similar trend was observed at day 47, with the pH of Al-add pots being lower (5.27–5.40) than that of no-Al pots (5.65–5.76). The y_1 values were significantly higher in Al-add (9.02–11.34) than no-Al (5.98–8.69) pots across the experiment.

3.2 | Regrowth of orchardgrass

The effect of fertilizer and acidification on the regrowth of orchardgrass varied across regrowth days (Table 3). On day 20, the dry weight of AGB and leaves increased by acidification in the CHE group ($p < 0.05$) but not in the other fertilizer treatments ($p > 0.1$, Table 3 and Figure 1). The dry weight of stubble increased by acidification in all fertilizer types ($p < 0.05$). On day 47, the acidification did not increase the dry weight of AGB regrowth ($p > 0.1$). Root regrowth was not affected by the acidification treatment on both sampling dates.

3.3 | Concentration of soil nutrients

Both fertilizer and acidification treatment affected the mineral concentration of the soil (Table 4). On day 20, IN, NO_3-N, and NH_4-N concentrations were significantly increased by adding aluminum sulfate in all fertilizer types ($p < 0.05$, Table 4 and Figure 2). The increment was greater in CHE and CMP than CNT and DNG on day 20. A similar trend was observed on day 47, with IN and NH_4-N concentration in CHE and CMP being higher in Al-add compared with no-Al (Figure 2). In addition, IN and NH_4-N concentrations in DNG with no-Al increased over the regrowth period.

The concentration of MgO was higher in DNG and CMP than other fertilizer treatments and was increased by the addition of ammonium sulfate in all the fertilizer treatments on day 20. However, on day 47, the addition of ammonium sulfate decreased MgO concentration in all the fertilizer treatments ($p < 0.05$, Table 4). Concentration of CaO in Al-add treatment significantly rose on day 20 ($p < 0.05$) and tended to increase on day 47 ($p = 0.07$). Acidification treatment did not significantly influence P_2O_5 and K_2O contents ($p > 0.1$).

4 | DISCUSSION

Orchardgrass persists poorly in acidic soils, and little research has investigated the effect of fertilization on the regrowth as well as the persistence of this species under acidic soil conditions. In the present study, we investigated the effect of fertilizer type on the concentration of soil nutrients and the regrowth of orchardgrass under different soil acidification levels.

Top-dressed application of aluminum sulfate to potting soil induced further soil acidification. This acidification increased the dry weight of AGB, which consisted of leaves and stubble, in the CHE treatment on day 20 (Table 3 and Figure 1), indicating that acidic soil encourages regrowth of orchardgrass with chemical fertilization at least in the early stages after fertilization. This result suggests that chemical fertilizers, the major type of fertilizer applied in hay meadows, increase the survivability of orchardgrass in strongly acidic soil under cutting conditions. The increase of dry weight of stubble and leaves by soil acidification is notable from the perspective of the persistence of orchardgrass. Stubble is an important part of the orchardgrass regrowth because carbohydrate reserves, primarily stored in the lower regions of stems, support initial regrowth after defoliation (White, 1973).

The encouraging orchardgrass regrowth observed in the CHE with acidification treatment is probably caused by high IN in the acidified potting soil. As shown in Table 4 and Figure 2, the soil content of IN, NO_3-N, and NH_4-N significantly increased on day 20 following soil acidification. Fertilized nitrogen is generally lost through denitrification, volatilization of ammonia, and leaching of NO_3-N from grassland soil; however, all of these forms of losses are decreased in acidic soil conditions (Aciego Pietri & Brookes, 2008; Müller et al., 1980). Water-soluble nutrients such as NO_3-N are leached out by excess water drained out of the potting soil. On the contrary, NH_4-N, which was not subject to leaching, is accumulated by nitrification inhibition in acidic soil (Aciego Pietri & Brookes, 2008; Kemmitt et al., 2005). Furthermore, orchardgrass absorbs and utilizes ammonium ions as well as nitrate ions (MacLeod & Carson, 1965). Therefore, our result suggests that soil acidification provides more available nitrogen for orchardgrass. A good supply of nitrogen is an important factor affecting orchardgrass leaf growth and its persistence (Van Santen & Sleper, 1996). Orchardgrass is a responsive species to nitrogen fertilizer and becomes very competitive to other species when nutrients are available (Jung & Baker, 1985). For this reason, the increase of soil IN may increase the survivability of orchardgrass under strongly acidic soil conditions.

Despite significantly higher soil IN (Figure 2), AGB and leaves of orchardgrass did not increase in the CNT, DNG, and CMP treatments (Figure 1). As far as measured in the present study, no possible factor other than IN was found to be associated with a difference in the growth of orchardgrass between acidification treatments. These results suggest that the increase of soil IN in these treatments until day 20 is insufficient to promote orchardgrass regrowth. Saarijarvi & Virkajarvi (2009) showed that chemical fertilizer increased soil IN substantially within a couple of days, whereas soil IN underneath cattle dung pats was highest on days 21 and 49. Application of organic manure, such as cattle manure compost, usually results in temporal immobilization of IN in the soil (Whitehead, 1995). Our result that IN and NH_4-N concentration in the DNG with no-Al increased over the regrowth period also suggests that a high concentration of IN is not maintained during the regrowth period.

The Al-add treatment, regardless of fertilizer type, temporarily increased CaO and MgO on day 20 (Figure 3), which was inconsistent with previous studies that demonstrated decreases in these cations with soil acidification (Bojórquez-Quintal et al., 2017; Martins et al., 2014). In this study, top-dressed aluminum sulfate induced an increase of NH_4-N. According to Di & Cameron (2004), the loss of cations from the soil is mainly due to NO_3-N leaching and is reduced by nitrification inhibitors. Therefore, nitrification inhibition by soil acidification may have temporarily suppressed cation leaching from the soil during the experimental period.

As previous studies show that application of cattle dung ameliorates acid soil (During et al., 1973; During & Weeda, 1973), it was anticipated that reduction of soil acidification by grazing cattle dung would be an effective factor in varying orchardgrass regrowth. However, soil acidification was observed regardless of fertilizer type in our study, indicating that soil amendment effects of grazing cattle dung are very restricted underneath or very close to thick dung pats (Yoshitake et al., 2014), and does not explain why regrowth of orchardgrass is altered under different soil acidification levels.

5 | CONCLUSIONS

The regrowth of orchardgrass was increased by soil acidification with the application of chemical fertilizer, possibly due to the high concentration of inorganic nitrogen in the acidified soil. Although soil acidification also increased the concentration of inorganic nitrogen in the soil that applied cattle manure compost and grazing cattle dung, these fertilizers did not substantially increase the regrowth of orchardgrass under strongly acidic soil conditions. The different regrowth rates of orchardgrass depending on the type of fertilizer applied suggests that the fertilizer application regime is one of the factors contributing to the persistence of orchardgrass in strongly acidic soil under cutting conditions.

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TABLE 1 Chemical composition of potting soil, chemical fertilizer (CHE), grazing cattle dung (DNG), and cattle manure compost (CMP) at the beginning of the experiment

Component	Potting soil (per kg dry soil)	CHE (per pot)	DNG (per pot)	CMP (per pot)
Moisture (%)	-	-	87.5	46.5
TC (mg)	-	-	595	486
TN (mg)	5200	42.0	33.5	31.2
NO_3-N (mg)	6.1	-	0.01	2.01
NH_4-N (mg)	5.9	-	0.09	0.18
P_2O_5 (mg)	682	34.0	33.3	29.1
K_2O (mg)	383	34.0	14.4	49.6
CaO (mg)	1080	-	19.9	23.7
MgO (mg)	151	-	19.2	15.8
C/N ratio	-	-	17.77	15.57
pH	5.20	3.77	8.04	7.35

TABLE 2 Soil pH and exchangeable acidity (y_1) in each treatment on Day 20 and Day 47

Measurement item	CNT†		CHE		DNG		CMP		RMSE	Effect of treatment		
	no-Al‡	Al-add	no-Al	Al-add	no-Al	Al-add	no-Al	Al-add		Fertilizer	Acidification	Interaction
Day 20												
pH	5.61	5.16	5.61	5.07	5.63	5.15	5.63	5.09	0.09	NS	$p < 0.05$	NS
y_1	7.69	9.55	8.69	11.34	8.07	9.42	7.56	9.89	1.14	NS	$p < 0.05$	NS
Day 47												
pH	5.76	5.31	5.65	5.27	5.75	5.40	5.73	5.36	0.11	NS	$p < 0.05$	NS
y_1	6.32	9.66	7.49	10.63	5.98	9.16	6.66	9.02	1.16	NS	$p < 0.05$	NS

Results are presented as estimated marginal means with root mean square error (RMSE).

† CNT, unfertilized control; CHE, chemical fertilizer; DNG, grazing cattle dung; CMP, cattle manure compost.

‡ no-Al, only water was added; Al-add, aluminum sulfate hydrate was added as a water solution to induce further soil acidification.

TABLE 3 Growth of orchardgrass in each treatment on Day 20 and Day 47

Growth parameters	CNT†		CHE		DNG		CMP		RMSE	Effect of treatment		
	no-Al‡	Al-add	no-Al	Al-add	no-Al	Al-add	no-Al	Al-add		Fertilization	Acidification	Interaction
Day 20												
AGB §	0.09	0.13	0.11	0.28	0.11	0.11	0.09	0.15	0.05	$p < 0.05$	$p < 0.05$	$p < 0.05$
Leaves	0.02	0.02	0.03	0.08	0.02	0.02	0.02	0.04	0.01	$p < 0.05$	$p < 0.05$	$p < 0.05$
Stubble	0.07	0.11	0.08	0.20	0.08	0.09	0.06	0.12	0.04	NS	$p < 0.05$	NS
Roots	0.16	0.17	0.17	0.25	0.17	0.16	0.18	0.18	0.05	NS	NS	NS
Day 47												
AGB	0.18	0.17	0.18	0.22	0.13	0.14	0.12	0.11	0.04	$p < 0.05$	NS	NS
Leaves	0.05	0.04	0.06	0.07	0.03	0.03	0.02	0.03	0.01	$p < 0.05$	NS	NS
Stubble	0.13	0.13	0.12	0.16	0.10	0.11	0.09	0.08	0.03	$p < 0.05$	NS	NS
Roots	0.25	0.23	0.24	0.30	0.19	0.19	0.15	0.19	0.06	$p < 0.05$	NS	NS

Results are presented as estimated marginal means with root mean square errors (RMSE). † CNT, unfertilized control; CHE, chemical fertilizer; DNG, grazing cattle dung; CMP, cattle manure compost. ‡ no-Al, only water was added; Al-add, aluminum sulfate hydrate was added as a water solution to induce further soil acidification. § AGB, Aboveground biomass.

TABLE 4 Soil nutrients in each treatment on Day 20 and Day 47

Soil nutrients	CNT†		CHE		DNG		CMP		RMSE	Effect of treatment		
(per kg dry soil)	no-Al‡	Al-add	no-Al	Al-add	no-Al	Al-add	no-Al	Al-add		Fertilizer	Acidification	Interaction
Day 20												
TC (g)	75.1	76.7	76.3	76.9	76.4	79.3	79.1	76.7	3.8	NS	NS	NS
TN (g)	5.0	5.2	5.1	5.2	5.2	5.4	5.4	5.2	0.3	NS	NS	NS
IN (mg)	10.9	15.8	13.8	28.7	12.1	19.2	15.1	29.8	2.1	$p < 0.05$	$p < 0.05$	$p < 0.05$
NO_3-N (mg)	3.9	5.1	3.9	7.3	4.5	5.9	4.4	7.1	1.1	$p = 0.07$	$p < 0.05$	NS
NH_4-N (mg)	7.1	10.7	9.9	21.5	7.7	13.4	10.6	22.8	1.9	$p < 0.05$	$p < 0.05$	$p < 0.05$
P_2O_5 (mg)	795	795	777	803	793	820	807	799	46	NS	NS	NS
K_2O (mg)	225	244	221	201	244	246	293	286	19	$p < 0.05$	NS	NS
CaO (mg)	1052	1287	1216	1355	1119	1381	1193	1431	72	$p < 0.05$	$p < 0.05$	NS
MgO (mg)	147	172	148	166	161	183	160	182	11	$p < 0.05$	$p < 0.05$	NS
Day 47												
TC (g)	75.6	74.8	74.8	76.9	75.1	74.7	72.4	75.8	3.6	NS	NS	NS
TN (g)	5.1	5.0	5.0	5.1	5.1	5.0	4.8	5.1	0.2	NS	NS	NS
IN (mg)	12.3	7.8	8.7	16.9	16.9	12.6	12.0	17.5	2.7	$p < 0.05$	NS	$p < 0.05$
NO_3-N (mg)	1.2	1.4	1.2	0.8	1.3	1.1	1.0	1.4	0.4	NS	NS	$p = 0.08$
NH_4-N (mg)	11.2	6.5	7.5	16.2	15.6	11.5	11.1	16.1	2.8	$p < 0.05$	NS	$p < 0.05$
P_2O_5 (mg)	768	761	742	731	731	758	724	773	27	NS	NS	NS
K_2O (mg)	228	216	197	196	235	215	249	258	15	$p < 0.05$	NS	NS
CaO (mg)	1099	1128	1080	1157	1085	1113	1089	1148	71	NS	$p = 0.07$	NS
MgO (mg)	157	129	143	110	161	132	146	135	15	$p = 0.07$	$p < 0.05$	NS

Results are presented as estimated marginal mean with root mean square error (RMSE). † CNT, unfertilized control; CHE, chemical fertilizer; DNG, grazing cattle dung; CMP, cattle manure compost. ‡ no-Al, only water was added; Al-add, aluminum sulfate hydrate was added as a water solution to induce further soil acidification.

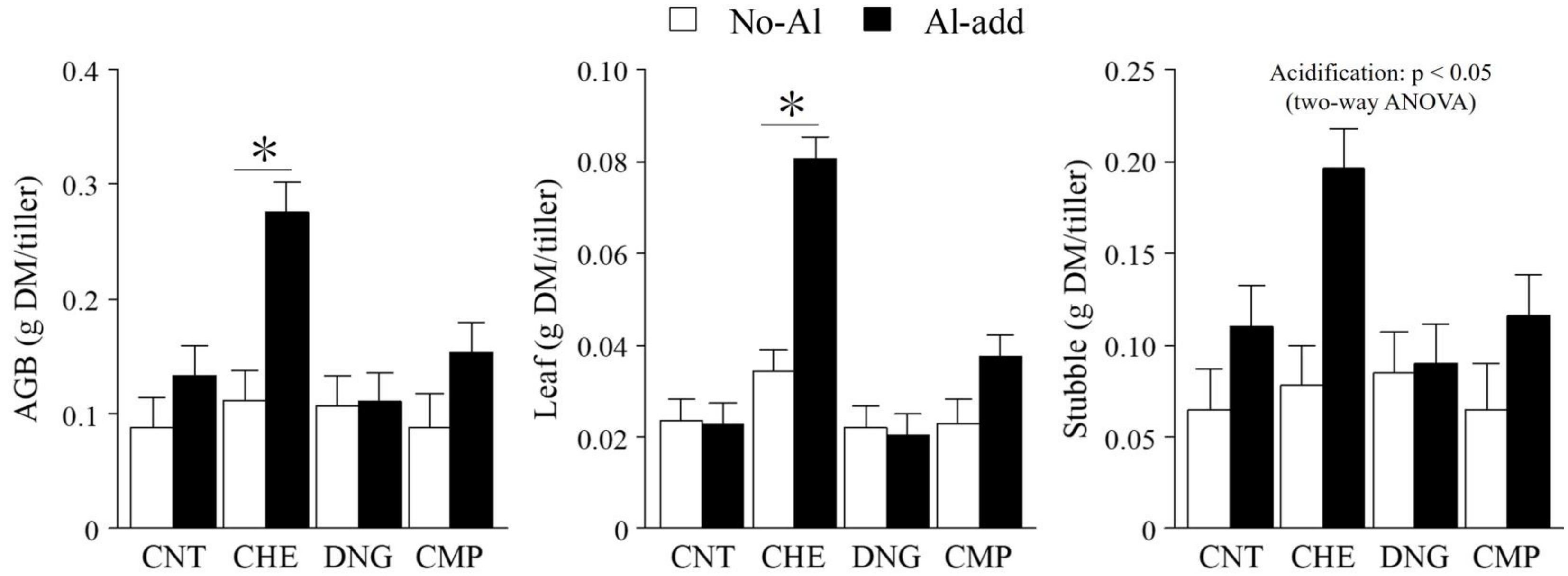

FIGURE 1 Dry weight of aboveground biomass (AGB), leaves, and stubble of orchardgrass in each treatment (estimated marginal mean + standard error) on day 20 after fertilization. Asterisks (*) indicate a significant difference between no-Al and Al-add within each fertilizer treatment (two-way ANOVA and pairwise multiple comparisons with Holm's method, $p < 0.05$).

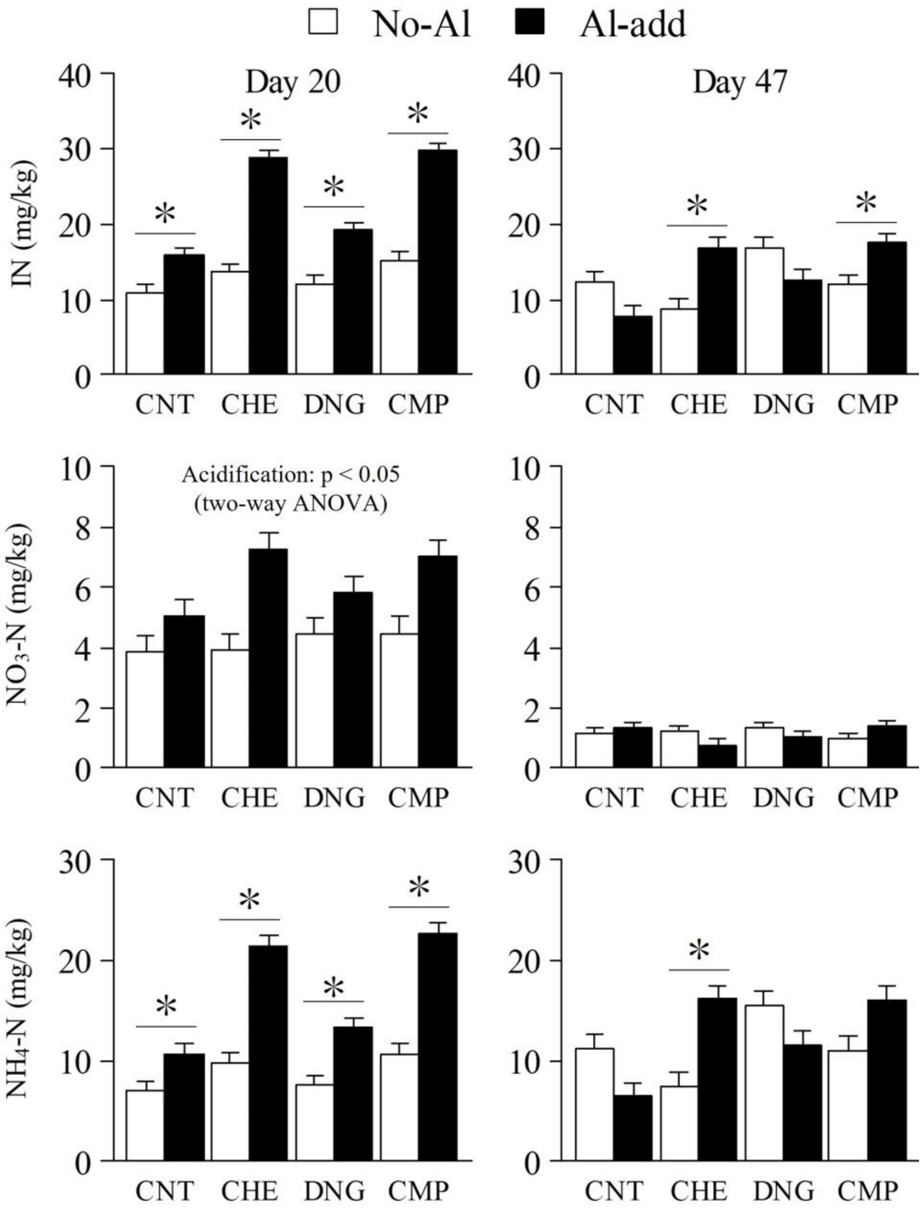

FIGURE 2 Inorganic nitrogen (IN), nitrate-nitrogen (NO_3-N), and ammonium-nitrogen (NH_4-N) in dry soil of each treatment (estimated marginal mean + standard error) on day 20 (left) and day 47 (right) after fertilization. Asterisks (*) indicate a significant difference between no-Al and Al-add within each fertilizer treatment (two-way ANOVA and pairwise multiple comparisons with Holm's method, $p < 0.05$).