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Winter cover crop integration on soil nitrogen loss from a subtropical warm-season pasture field and its potential mitigation

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Abstract

Bermudagrass is a summer forage crop with high nitrogen (N) demand. Introducing winter cover crops may sustain bermudagrass yields with less fertilizer, reducing environmental N losses. Field trials and lab incubation were carried out to evaluate the effects of white clover (*Trifolium repens* L.) and ryegrass (*Lolium multiflorum*) as winter cover crops on soil N losses through runoff and nitrous oxide (N_2O) emissions in a subtropical bermudagrass (*Cynodon dactylon* L.) pasture field. The 2-year field experiment included five treatments: (1) no winter cover and N fertilization as a control (CLT), (2) white clover without N fertilization (WC) (where WC represents white clover), (3) white clover mixed with ryegrass without N fertilization (WCR), (4) white clover with half-rate N at 112 kg N ha^{-1} $year^{-1}$ (WC112N), and (5) no cover crop with full-rate N 224 kg N ha^{-1} $year^{-1}$ (224N). Results showed that without N fertilization, WC increased bermudagrass biomass by 38% compared to CLT, while WCR had a similar bermudagrass biomass yield to CLT. WC112N produced comparable bermudagrass biomass as 224N as well as reduced NH_4^+ and NO_3^- runoff loss by 30%–35% and 11%–24%, respectively, compared than 224N, due to decreased runoff volume. There was no difference in N_2O emissions between 224N and WC112N. Laboratory incubation of white clover residue-amended soil showed

Abbreviations: ANOVA, analysis of variance; CLT, control; DCD, dicyandiamide; DMPP, 3,4-dimethylpyrazole phosphate; GC, gas chromatography; NBPT, *N*-(*n*-butyl) thiophosphoric triamide; WC, white clover; WCR, white clover–ryegrass mixture; WFPS, water-filled pore space.

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that nitrification inhibitors dicyandiamide and 3,4-dimethylpyrazole phosphate lowered N_2O emission significantly, with a maximum reduction of 77%–91%, while urease inhibitor *N*-(*n*-butyl) thiophosphoric triamide had no effect. Overall, clover incorporation during the winter season helps in developing a low-N-input pasture production system, and nitrification inhibitors could be applied to mitigate associated N_2O losses from clover residue decomposition.

Plain Language Summary

Field trials were carried out to evaluate the effects of white clover and white clover–ryegrass mixture as winter cover crops on soil nitrogen losses via runoff and nitrous oxide emission in a subtropical bermudagrass pasture field. Results showed that white clover treatment increased bermudagrass biomass yields, while white clover–ryegrass mixture treatment did not compared to the control. White clover combined with half of the recommended full fertilizer nitrogen rate produced comparable bermudagrass biomass as the full fertilizer nitrogen rate treatment and also reduced surface runoff losses of ammonium and nitrate. Laboratory incubation experiment showed that nitrification inhibitors lowered N_2O emissions significantly, while the urease inhibitor had no effect. Overall, clover incorporation during the winter season helps in developing a low-nitrogen-input pasture production system, and nitrification inhibitors could be applied to mitigate associated N_2O losses from clover residue degradation.

1 | INTRODUCTION

Warm-season perennial bermudagrass (*Cynodon dactylon* L.) is the primary species that provides forage grazing or hay harvesting for livestock production in the southern United States. The bermudagrass pasture normally has a short growth season, from April through September, and it requires a high soil nitrogen (N) supply (Han et al., 2012). Within the southern United States, common N fertilization rates range from 135 to 336 kg ha^{-1} per single season for bermudagrass hay production (Duble, 1996; Redfearn et al., 2016). Due to known drawbacks of mineral N fertilizers associated with high cost and environmental pollution, there has been increasing interests in developing low-input sustainable agriculture systems in the United States (Lüscher et al., 2014; Meena et al., 2018; Tian et al., 2021). Forage legumes, such as clover, have the potential to mitigate environmental issues by reducing reliance on mineral N fertilizers (Franzluebbers et al., 2014; Pena-Yewtukhiw et al., 2017). In addition, legume cover crops enhance soil health and promote sustainable production by enriching soil nutrients, improving soil structure, and increasing organic matter content (Koudahe et al., 2022; Meena et al., 2018).

Clover species have been evaluated for their adaptability, yield, and quality under various conditions. Among them, Durana white clover (WC) (*Trifolium repens* L.) developed from Georgia ecotypes demonstrates superior persistence in southern US tall pastures compared to other traditional Ladino cultivars, with its significantly higher stolon density resulting in greater dry matter production (Bouton et al., 2005). A pure stand of WC pasture can contribute 55–296 kg N ha^{-1} $year^{-1}$ under grazed conditions (Ledgard & Steele, 1992). In a mixed legume/grass pasture, bio-fixed N from legumes can transfer to associated grasses through root exudation and decomposition of foliage, nodules, and roots (Ledgard & Steele, 1992). Currently, there is little information about WC N transfer rate and fertilizer replacement ability as a winter cover crop in bermudagrass pasture. Limited studies have focused on WC N transfer rate within bermudagrass grown as turfgrass, which is different from hay production that regularly removes biomass (McCurdy et al., 2013, 2014; Sincik & Acikgoz, 2007). According to McCurdy et al. (2014), WC fixed 66 kg N ha^{-1} $year^{-1}$ and contributed 24% of total bio-fixed N to warm-season turfgrass in the southeastern United States. Sincik & Acikgoz (2007) showed that WC fixed more than 250 N ha^{-1} $year^{-1}$, but only 4.2%–13.7% of that N transferred to cool-season turfgrass. In general, the bio-fixed N contribution and transfer rate varied, depending on how well legume/grass systems synchronize bio-fixed N mineralization and N demands of grass (McCurdy et al., 2013).

Annual ryegrass (*Lolium multiflorum* Lamarck), a cool-season grass, is commonly overseeded with WC as a companion grass during the winter season. Research has shown that overseeding ryegrass into winter cover crops may improve synchronization between the N mineralization of winter-grown biomass and the N demands of summer grass (Kuo & Jellum, 2002). When grown with WC, ryegrass actively captures excess soil NO_3^- during the cool season, significantly reducing N leaching losses by 24%–54% compared to other pasture species (Andrews et al., 2007; Malcolm et al., 2015). As temperatures rise and ryegrass residues decompose, the N taken up by ryegrass can be gradually mineralized and become available to meet the N demands of subsequent summer grass (Kuo & Jellum, 2002).

Similar to mineral N fertilizers, legume cover crops benefit the growth of bermudagrass by supplying bio-fixed N. However, during cover crop decomposition, the released N can be lost through various pathways, including NO_3^- runoff and leaching, and nitrous oxide (N_2O) emissions (Hochman & Helyar, 1989; Ledgard et al., 1999). N_2O is a potent greenhouse gas with a global warming potential 265 times greater than that of CO_2 (Myhre et al., 2013). Several studies have compared the environmental impacts of bio-fixed N with mineral fertilizers. Studies by Li et al. (2011) found that WC/ryegrass-N released 19.6% less N_2O to the atmosphere than mineral N-fertilized ryegrass. However, other studies reported that legume residues had the same N_2O emission factor as urea (Gomes et al., 2009). Despite these conflicting results, few studies have considered total N input and biomass productivity while comparing N loss from different N sources. Moreover, existing studies have demonstrated that when mineral N fertilizer and legume plant residues as green manure were applied to a field, it was the total N input, rather than the fertilizer type, that primarily determined the amount of N loss (Chapman et al., 2018; Ledgard et al., 2009; Sprosen et al., 1997). Currently, there has been no systematic investigation on legume cover crop-induced N loss through N_2O emissions and water runoff as well as the impact of legume on the partial substitution of mineral N for hay production.

Among various technologies available to control N losses from soil-applied fertilizers, N stabilizers such as dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP) that serve as nitrification inhibitors were shown to significantly reduce N_2O emissions (Di & Cameron, 2006; Meng et al., 2021; Misselbrook et al., 2014). Additionally, urease inhibitors such as *N*-(*n*-butyl) thiophosphoric triamide (NBPT) were found to effectively slow down the hydrolysis of urea and subsequently minimize N loss from volatilization and oxidation to nitrate (Liu et al., 2017; Tian et al., 2015). Plant residue also releases N_2O after undergoing a series of biochemical reactions in soil: mineralization, nitrification, and denitrification (Basche et al., 2014; Velthof et al., 2002). However, only limited studies on N stabilizer effects have been conducted on plant-derived N, which focused on the influence of nitrification inhibitors on N_2O emission from grass–clover residues and vegetable crop residues (Chaves et al., 2006; Nair et al., 2020). Therefore, future research is necessary to evaluate the inhibition efficiency of DCD, DMPP, and NBPT on N_2O emissions from WC as a potential N source.

Core Ideas

- Substituting 50% mineral N fertilizer with white clover gave comparable bermudagrass yield.
- Clover-substituted plots had lower N runoff loss but similar N_2O emissions to mineral N application.
- Clover–ryegrass mixtures had similar N runoff loss but lower bermudagrass yield than pure clover.
- Nitrification inhibitors reduced N_2O emission from the decomposition of clover residue.

The objectives of this study were as follows: (1) determine the potential to substitute mineral N fertilizer with cool-season WC as a cover crop on pasture production; (2) evaluate combined N inputs of mineral N fertilizer, WC, and ryegrass on the loss of N through runoff and N_2O emissions; and (3) investigate the efficiency of N stabilizers in minimizing N_2O emission from WC mineralization under a humid subtropical climate that is highly conducive to organic matter mineralization and N losses.

2 | MATERIALS AND METHODS

2.1 | Field experiment

2.1.1 | Field site properties

A 2-year field experiment was conducted from Fall 2016 through Fall 2018 in a pasture field in Baker, LA (30°36′14″ N latitude, 91°10′5″ W longitude). The annual average temperature was 20°C, and the annual average precipitation was 1570 mm. The site's daily mean air temperature and precipitation during measurements were presented in Figure S1. Prior to the experiment, the field was a bermudagrass pasture during summer, with less than 20% of other weeds, primarily carpetgrass (*Axonopus fissifolius* (Raddi) Kuhlm) and bahiagrass (*Paspalum notatum* Flugge). The field was kept unfertilized and non-grazed for up to 5 years. The soil at this site is classified as Oprairie silt (fine-silty, mixed, semiactive, thermic Fragiaquic Glossudalfs), with a texture composed of 13.0% clay, 72.7% silt, and 14.3% sand. Additional soil physical and chemical properties are provided in Table S1.

At the beginning of the study, the soil had a total N content of 1.69 g kg^{-1} and a total C content of 14.33 g kg^{-1}. The KCl-extractable NO_3^- and NH_4^+ were 1.20 and 2.17 mg N kg^{-1}, respectively (Table S1). The field was maintained following common practices of Bermudagrass hay production in Louisiana (Han & Twidwell, 2017). A blanket application of P_2O_5 (54 kg ha^{-1}) and K_2O (80 kg ha^{-1}) was carried out each year in late October or early November after harvesting the biomass based on the recommendation of the Louisiana State University Agricultural Center Soil Testing Laboratory.

2.1.2 | Treatments

Experimental plots were established to evaluate the effects of winter cover crop inclusion and supplemental N rates on warm-season bermudagrass yields and N loss. The experiment included five treatments: (1) control (CTL) with no winter cover crop and 0 N rate (CLT), (2) white clover (WC) as winter cover and 0 N rate (WC), (3) WC and ryegrass mixture as winter cover crop and 0 N rate (WCR), (4) WC as winter cover crop and the bermudagrass received 112 kg N ha^{-1} $year^{-1}$ as urea (WC112N), and (5) no winter cover crop was planted and the bermudagrass received 224 kg N ha^{-1} $year^{-1}$ as urea (224N). Treatments were arranged in a randomized block design, with each plot measuring 10 m^2 in area (4 m × 2.5 m), and a 2-m buffer zone between the plots. Winter cover crop species, Durana WC and annual ryegrass, were overseeded in preexisting bermudagrass through broadcasting in October of 2016 and 2017. The seed rates were 4.5 kg ha^{-1} for WC and 33.6 kg ha^{-1} for annual ryegrass. The urea treatments were split, with 50% applied in late May/early June and the remainder in mid-August, approximately 50 days before hay harvest. All field treatments were replicated four times. Gas emission, runoff, and leachate were monitored right after each N fertilization event.

2.1.3 | Aboveground herbage yield

The winter cover crops (WC and white clover–ryegrass mixture) were allowed to naturally senesce as the summer season progressed. To estimate N input from the winter cover crops, the aboveground biomass in WC and WCR plots was sampled from a 1-m^2 quadrat in April, when the cover crops reached peak growth. For bermudagrass, aboveground biomass was manually harvested twice during each growth season by mowing down to 5-cm stubble height. Harvest dates were July 10 and September 26 in 2017, and July 20 and September 20 in 2018. To estimate bermudagrass yield, the aboveground biomass from a 1-m^2 quadrat in each plot was also sampled.

Plant biomass was dried in a forced-air oven at 50°C. The dried biomass was ground and analyzed for total C and N using a Vario EL cube Elemental Analyzer (Elementar Co.) via combustion. The aboveground N yield of bermudagrass was calculated by multiplying the herbage dry mass by the total N contents of the herbage. The aboveground N input from the winter cover crop was calculated by multiplying the herbage dry mass by the total N contents of the herbage. The total (above- and belowground) biomass N input from winter cover crops was estimated by multiplying the aboveground biomass N input by correction factors ranging from 1.7 to 2.3, accounting for root-derived N input (Jørgensen & Legard, 1997; Talgre et al., 2017). Nitrogen transfer from winter cover crops to bermudagrass was determined by subtracting the N yield of bermudagrass in CLT plots from the N yield of bermudagrass in WC and WCR plots (McCurdy et al., 2014).

2.1.4 | N_2O emissions

N_2O emissions were measured after each of two split N fertilizations till herbage harvest (two harvests each season) based on a modified closed chamber method (Bekku et al., 1995; Meng et al., 2021). Specifically, the monitoring periods were from May 26 to July 4 and from August 14 to September 24 in 2017, and from June 4 to July 18 and from August 7 to September 18 in 2018. During each monitoring period, gas samples were collected every other day in the first 2 weeks and then decreased to every 3–4 days during the later period. Chambers with a headspace volume of 25.75 L were installed and duplicated in each plot. For each sampling, 15 mL headspace gas samples were collected into pre-vacuumed vials at 0 min, 30 min, and 60 min after the chamber was closed, and transported to the laboratory for analysis (Tian et al., 2015). The N_2O concentrations were measured by using a Varian 3800 gas chromatography (GC) (Varian Inc.) equipped with a capillary column (27.5 m × 0.53 mm) and an electron capture detector. The injector, methanizer, and detector temperatures were kept at 60°C, 450°C, and 250°C, respectively. The GC was calibrated using N_2O standards (Scotty Gas). At each gas sampling, a soil sample (0- to 15-cm depth) was taken and measured for water content by oven-drying at 105°C, and the water-filled pore space (WFPS) was calculated based on gravimetric water content, bulk density, and particle density (Linn & Doran, 1984). Soil temperature (0- to 15-cm depth) was measured using a thermometer. In 2018, soil samples (0- to 15-cm depth) were periodically collected during gas sampling events to monitor NH_4^+ and NO_3^- dynamics. These samples were extracted with 2 M KCl and analyzed, with the results presented in Figure S2. The cumulative N_2O emissions from each plot were calculated by linear interpolation of the measured daily fluxes (Meng et al., 2021). The seasonal N_2O emission factor was calculated using the following equation (Tian et al., 2015):

Emission factor = (cumulative N_2O emissions from fertilized or cover crop treatment − cumulative N_2O emissions from CTL) ÷ total N input from fertilizer or cover crops.

2.1.5 | Inorganic N in runoff

Inorganic N fractions (NH_4^+ and NO_3^-) losses through runoff were monitored throughout the experiment. Runoff water samples were collected after each rain event by installing a stainless metal frame collection system (a square with 60 cm side length and 20 cm in height) at the edge of each plot (Gaston et al., 2003). The collection system contains a trough (10 cm wide) on one side of the collection frame, and the trough was orientated toward the downslope side of each plot so that the other three sides of the frame served as barriers to converge surface runoff water into a 30-L water collection chamber that was buried at the side of each plot. During rain events, runoff water collected in the frame area was drained into the collection chamber by gravity. Runoff volumes were measured using a graduated cylinder, and water subsamples were taken from the collection chamber after each rain event.

Runoff water samples were filtered through 0.45-μm polytetrafluoroethylene membrane syringe filters (VWR International LLC) and analyzed for NH_4^+ and NO_3^- concentrations using a LaChat flow injection system with inline colorimetry (LaChat Instruments). The cumulative load of NH_4^+ and NO_3^- in runoff water was calculated by summing the individual load of each rainfall event (concentration of NH_4^+ or NO_3^- in runoff × runoff volume) per area.

2.2 | Laboratory incubation

A laboratory incubation experiment was also carried out based on the outcome of the field experiment (Section 2.1), which revealed that N_2O emissions were associated with the decomposition of winter cover crop residues. The purpose of this controlled laboratory incubation was to investigate if N-stabilizers could be potentially used to mitigate N_2O emissions during residue degradation of winter cover crops. To accomplish this, soil was collected from the surface layer (0–15 cm) of non-fertilized plots, with no winter cover crops grown of the same field trial site, maintained at field-moist conditions, and passed through a 4-mm sieve to remove roots, surface litter, and rocks. The clover cover crop biomass harvested from the WC plots of the field trial site in April when WC was the dominant species was also used for the laboratory incubation experiment. The harvested clover biomass was dried at 50°C, ground to <5-mm particles, and then mixed homogeneously with the soil to set up the incubation.

Laboratory incubation consists of six treatments including (1) soil CTL (a soil without clover biomass or N stabilizers), (2) clover (soil mixed with WC biomass), (3) clover DCD 10% (soil mixed with WC biomass and 10% DCD), (4) clover DCD 100% (soil mixed with WC biomass and 100% DCD), (5) clover DMPP 10% (soil mixed with WC biomass and 10% DMPP), and (6) clover NBPT (soil mixed with WC biomass amendment and NBPT). In doing so, 30 g (oven-dry based) of moist soil and 2.8 g of clover aboveground biomass (equivalent to 224 kg clover-N ha^{-1}) were mixed in a modified Mason jar with the lid containing rubber gas sampling septa. Soil–clover mixtures were pre-incubated for 5 days at room temperature (22°C) under field capacity (66% gravimetrical soil moisture). All N stabilizers were applied by dissolving them in water followed by spraying the soil surface. The DCD (Sigma-Aldrich Corp.) was added at 10% and 100% wt./wt. of amended clover-N content (equivalent to 22.4 and 224 kg ha^{-1}, respectively). The DMPP (Sigma-Aldrich Corp.) was added at 10% wt./wt. of amended clover-N content (equivalent to 22.4 kg ha^{-1}). The rationale for both DCD rates of 10% and 100% was based on previous studies showing that DCD typically requires 10 times the application rate of DMPP to achieve comparable nitrification inhibition (Di & Cameron, 2006; Meng et al., 2021; Tian et al., 2015). The NBPT (Agrotain Ultra, 26.7% wt./wt. NBPT, Koch Agronomic Services, LLC.) was added at 0.2% wt./wt. of amended clover-N content (equivalent to 0.448 kg ha^{-1}). NBPT 0.2% and DCD 10% were the recommended rates for field use (Di & Cameron, 2006; Tian et al., 2015; Weiske et al., 2001). However, to obtain the maximum reduction and to compare to the regular DCD rates at 10%, DCD 100%, and DMPP 10% (10 times of their recommended rates) were applied. The incubation experiment was conducted over 20 days with jars open to the atmosphere. The gravimetrical soil moisture was adjusted to 66% (WFPS was 100% based on bulk density) at the beginning of incubation, then the system was allowed to undergo two wetting–drying cycles to simulate field rain events. Gas samples for N_2O from the incubation jars were collected after a 1-h closure period. N_2O concentrations were measured by using a Varian 3800 GC (Varian Inc.).

2.3 | Statistical analyses

Treatment effects on harvested biomass and cumulative N_2O emission were analyzed using one-way analysis of variance (ANOVA) and the GLIMMIX procedure of SAS software version 9.4 (SAS Institute, Inc.). When ANOVA test was significant, individual treatment means were tested using Tukey's least significant difference at the $\alpha < 0.05$ level.

3 | RESULTS AND DISCUSSION

3.1 | Pasture biomass and N yield

The dry biomass yield was similar between 2017 and 2018 under each treatment (Figure 1a), and N yield was generally proportional to the associated biomass yield (Figure 1b). Bermudagrass under WC treatment yielded an average of 38% more biomass than CLT treatment, resulting in 32–62 kg ha^{-1}

TABLE 1 N input from winter cover crops and N transferred to summer bermudagrass.

Treatment	Year	Aboveground biomass N input (kg ha^{-1})	Total biomass N input (estimated range) (kg ha^{-1})	N transferred to bermudagrass (kg ha^{-1})
WC	2017	53.3	90.6–122.6	31.8
	2018	56.3	95.7–129.5	62.2
	Two-year average	55.0	93.5–126.5	47.0
WCR	2017	51.9	88.2–119.4	29.7
	2018	54.2	92.1–124.7	22.6
	Two-year average	53.0	90.1–121.9	26.2

Note: The total (above- and belowground) biomass N input from cover crops was estimated by multiplying the aboveground biomass N input by correction factors ranging from 1.7 to 2.3, accounting for root-derived N input (Jørgensen & Legard, 1997; Talgre et al., 2017).
Abbreviations: WC, white clover; WCR, white clover with ryegrass.

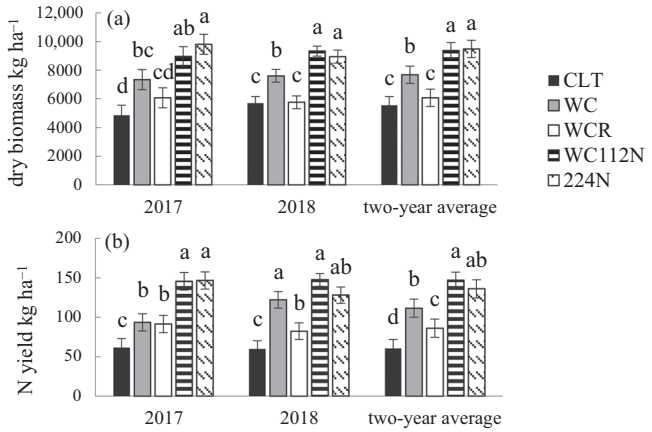


FIGURE 1 Treatment comparison of warm-season pasture for (a) biomass yield (kg ha^{-1}) and (b) N yield (kg ha^{-1}) over the 2017 and 2018 seasons. Bars indicate standard errors among replicates. Letters above the bars indicate significance among treatments using Tukey's least significant difference (LSD) comparison ($\alpha < 0.05$). 224N, 224 kg urea-N ha^{-1}; CLT, control; WC, white clover; WC112N, white clover with 112 kg urea-N ha^{-1}; WCR, white clover with ryegrass.

additional N being produced. The 2-year average yield in WC112N treatment was 9364 kg ha^{-1}, which was not significantly different from 9478 kg ha^{-1} in 224N treatment. The N yield of WC 112N was slightly higher than 224N; however, they were not statistically different. It is also interesting to note that the mixed WCR treatment produced an average of 29% less biomass and N than the WC treatment. This might be attributed to lower soil N availability, partly due to less biological N_2 fixation from clover's lower seeding rate and partly due to less N released from decomposing ryegrass biomass.

Winter cover crops in the WC and WCR treatments provided an average of 55.0 and 53.0 kg N ha^{-1} in their shoots, respectively (Table 1). Besides the aboveground portion, the decomposition of WC/ryegrass's roots and root excretion is considered a significant N source to the summer growing season (Legard & Steele, 1992). Jørgensen and Legard (1997) estimated that the belowground root biomass of WC contains 0.7 times of the aboveground biomass. A recent study reported that the belowground roots portion could contain up to 1.3 times more N than the shoots portion (Talgre et al., 2017). Using correction factors of 1.7–2.3, the WC was estimated to produce 93.5–126.5 kg N ha^{-1} in total biomass (above- and belowground), and the WC–ryegrass mixture produced 90.1–121.9 kg N ha^{-1} (Table 1).

3.2 | N transferred from winter cover crops to bermudagrass

Nitrogen transfer from cover crops to bermudagrass was estimated at 47.0 kg ha^{-1} in unfertilized WC plots and 26.2 kg ha^{-1} in WCR plots (Table 1). This corresponded to 40%–54% of the N derived from WC and 21.1%–28.5% from WCR, potentially contributing to subsequent summer hay yield. The transfer rate in WC was similar to the reported 41% from WC to bermudagrass without fertilizer in the subtropical southeastern region (McCurdy et al., 2014), and it was higher than 4.2%–13.7% of a cool-season legume–turfgrass system (Sincik & Acikgoz, 2007). The higher transfer rate found in this study may indicate higher N availability from fixed N in soils under relatively warmer environments.

The N yield of bermudagrass was proportional to its biomass yield, indicating that the different combinations of cover crops and fertilization rates would not affect the N content of biomass produced (Figure 1). Han et al. (2012) also found that bermudagrass overseeded with WC yielded a similar amount of N contents compared to bermudagrass that received 112 kg N ha^{-1} fertilizer under similar growing conditions. Similar biomass yield between WC112N and 224N treatments also agreed well with a study conducted in Texas that the amount of N fertilizer required by the bermudagrass to replace the WC was about 127 kg N ha^{-1} (Evers, 1985). These results supported that substituting 50% of N with WC was able to provide enough bio-fixed N for the growth of subsequent summer grass without compromising yield and N yield.

The ryegrass inclusion appeared to hinder summer bermudagrass growth, with the mixed WCR treatment

yielding 29% less biomass than the WC treatment (Figure 1a). The same negative effect of ryegrass on yield was also reported in a New Zealand pasture (Malcolm et al., 2015). The mineralization of ryegrass biomass was slower than that of clover (Griffith et al., 2000). Due to this delayed mineralization, ryegrass likely failed to synchronize with the high N demand of bermudagrass during its rapid growth period in June. This hypothesis is supported by the lower soil NH_4^+ concentration observed in June in the WCR treatment (average 37.7 mg N kg^{-1} soil) compared to the WC treatment (average 51.3 mg N kg^{-1} soil) (Figure S2). The reduced N availability likely limited N uptake by bermudagrass during its peak growth period, explaining the substantially lower N transfer from cover crops to bermudagrass in WCR relative to WC. Future research is needed to evaluate the seeding rate of ryegrass as a companion grass for the purpose of avoiding competition and achieving a better synchronization with the subsequent crop.

3.3 | N_2O emission

Temporal variation in N_2O emissions over the 2 years was given in Figure 2. In 2017, the N_2O flux was small after the first fertilization but peaked significantly after the second fertilization (Figure 2a). The N_2O flux reached 183 and 103 g N ha^{-1} day^{-1} peaks for 224N and WC112N treatments at Day 4, respectively, then dropped to background level after 20 days of fertilization, whereas other non-fertilized treatments showed very low N_2O emission throughout the growth season of 2017 (Figure 2a). The cumulative N_2O emissions from the 224N treatment were significantly greater than those treatments that received half the amount of fertilizer or no fertilizer. There was no significant difference in cumulative N_2O emissions among CLT, WC, and WCR treatments. Overall, the 2017 seasonal accumulative N_2O-N emissions for the CLT, WC, WCR, WC112N, and 224N were 130, 140, 168, 647, and 1126 g N ha^{-1}, respectively (Figure 3). In 2017, winter cover crops contributed a negligible amount of N_2O emissions during the sampling periods, where the peaks of N_2O were primarily associated with mineral fertilizer applications.

The N_2O emission trends in 2018 showed a different pattern from that in 2017 (Figure 2b). Significant N_2O fluxes of fertilized treatments started 2 days after the fertilization events. The peaks of N_2O fluxes from all treatments were related to the periods of high soil moisture content. In 2018, the flux peaks persisted over a longer duration compared to 2017. Consequently, the cumulative N_2O emissions in 2018 were significantly higher in both the WC (1451 g N ha^{-1}) and WCR (1181 g N ha^{-1}) treatments than in CLT (375 g N ha^{-1}). However, these values remained lower than those from urea-fertilized treatments, which reached 2411 g N ha^{-1} in WC112N and 2336 g N ha^{-1} in 224N (Figure 3).

Substituting 50% of urea fertilizer with Duran WC resulted in average cumulative N_2O emission comparable to the 224N treatment over 2 years (1529 vs. 1731 g N ha^{-1}; Figure 3). The calculated 2-year average N_2O emission factor for winter cover crop-derived N in WC treatment was found to be 0.63%, statistically similar to the 0.64% emission factor found for fertilizer-N in 224N treatment. This result indicates that cover crop-derived N and urea-N have equivalent emission potentials. These findings align with previous studies on grazed pastures that reported similar N_2O emissions from legume-fixed N and fertilizer-N when applied at equivalent N rates (Aulakh et al., 2001; Ledgard et al., 2009). Moreover, consistent with our experimental approach where cover crops were allowed to terminate naturally, several studies have reported N_2O emissions from decomposing cover crop residues left on the soil surface without tillage incorporation (Baggs et al., 2003; Gomes et al., 2009; Millar et al., 2004). For example, Gomes et al. (2009) observed N_2O emission factors ranging from 0.39% to 0.75% during legume cover crop residue decomposition in no-till systems, close to the results of 0.63% in our study. While dying cover crops serve as a slow release of N_2O due to decomposition as suggested by others (Baggs et al., 2003; Millar et al., 2004), our results likely indicate a comparably rapid process of N_2O emission from the application of mineral fertilizer-N such as urea over the summer growth season of bermudagrass.

The result shows that cover crop-derived N and urea-A have equivalent emission potentials, highlighting that legume cover crops can be a significant source of N_2O. The N_2O emission from clover biomass could be attributed to several factors. The clover biomass had a low C:N ratio of 19.1 (Table S2), consistent with previous studies reporting that clover biomass typically has low C:N and lignin:N ratios that allow rapid mineralization (Brunetto et al., 2011; Huang et al., 2004; Kumar & Goh, 2002; McCurdy et al., 2013). The rapid mineralization was evident by the observed rapid release of NH_4^+ and NO_3^- substrates during clover decomposition in June (Figure S2). On the other hand, the rapid mineralization process depletes oxygen and creates anaerobic microsites known as hotspots of denitrification (Schlüter et al., 2019). Moreover, the decomposed biomass can increase soil labile C, which serves as the electron donor (Mitchell et al., 2013). The soil surface mulched by drying clover could also enhance soil moisture and temperature (Baggs et al., 2003). All these conditions brought by decomposing clover favor denitrification and stimulated N_2O production to as high as 76.4 g N ha^{-1} day^{-1} in mid-June.

Since N_2O emission is tightly coupled with total N input and plant N uptake (Legard et al., 2009), our result suggested that when trying to achieve a similar yield, bio-fixed N, as with WC, may not be an advantageous mitigation strategy for minimizing N_2O emission, especially in a subtropical grassland where frequent rain events happen during

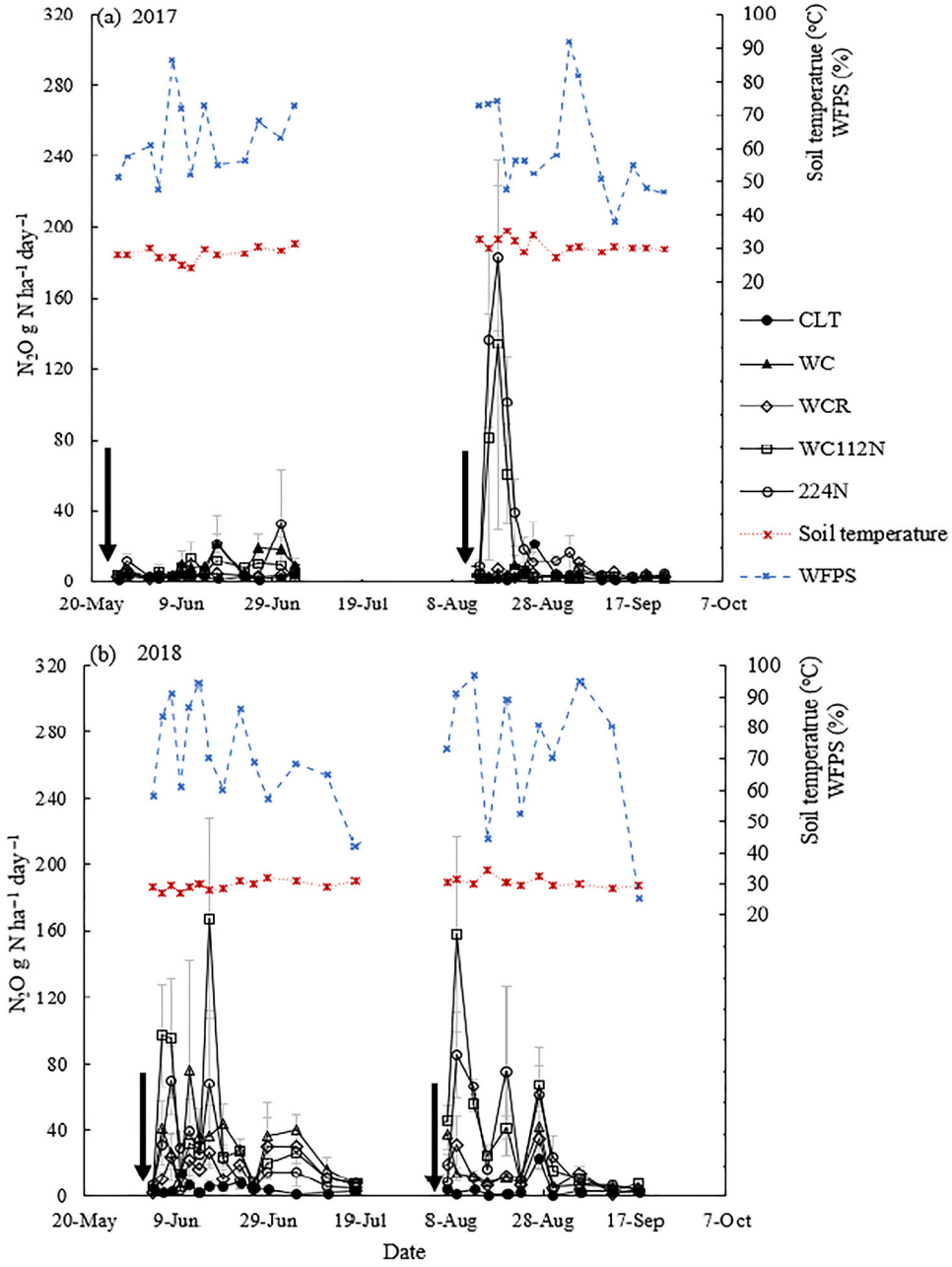


FIGURE 2 Temporal N_2O emission, soil temperature, and water-filled pore space (WFPS) during the growth season of (a) 2017 and (b) 2018. Arrows indicate the date of N fertilization. Bars indicate standard errors among replicates. 224N, 224 kg urea-N ha^{-1}; CLT, control; WC, white clover; WC112N, white clover with 112 kg urea-N ha^{-1}; WCR, white clover with ryegrass.

the decomposing period of legume residue. On the other hand, it is interesting to note that WCR emitted 21.9% less cumulative N_2O than WC on average over 2 years (Figure 3). Ryegrass can capture N released from clover (Laidlaw et al., 1996; Paynel et al., 2001; Šimek et al., 2004), while its own biomass mineralizes more slowly (Griffith et al., 2000), likely explaining the reduced N_2O emissions in WCR versus WC treatments. This finding suggests that incorporating ryegrass as a cover crop might be an effective strategy for the purpose of N_2O mitigation.

3.4 | Inorganic N loss via surface water runoff

Inorganic N losses via surface water runoff are presented as cumulative loadings in Figure 4. Summer N runoff loss was clearly influenced by winter cover crops and N fertilizer rates. Runoff N loss in fertilized treatments was significantly greater in both years. Ammonium-N loss from the 224N treatment accounted for 1.7%–2.4% of applied N fertilizer (5571 g N ha^{-1} in 2017 and 3783 g N ha^{-1} in 2018). Nitrate-N loss from

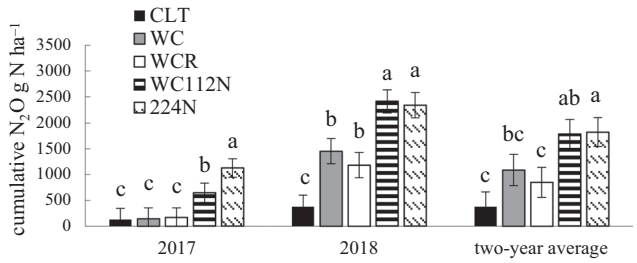


FIGURE 3 Cumulated N_2O emission in 2017, 2018, and the 2-year average. Letters above bars indicate significance among treatments using Tukey's least significant difference (LSD) comparison ($\alpha < 0.05$). Bars indicate standard errors among replicates. 224N, 224 kg urea-N ha^{-1}; CLT, control; WC, white clover; WC112N, white clover with 112 kg urea-N ha^{-1}; WCR, white clover with ryegrass.

224N accounted for 2.0%–3.1% of applied N fertilizer (5382 g N ha^{-1} in 2017 and 11,383 g N ha^{-1} in 2018). Runoff N loss from WC112N treatment was significantly less than from 224N, with 30%–35% and 11%–24% reduction in NH_4^+-N loss and NO_3^--N loss, respectively. Runoff N loss showed no significant difference between unfertilized winter cover crops treatments (1113 g N ha^{-1} on average) and CLT treatment (995 g N ha^{-1}) in 2017. However, in 2018, runoff N losses were significantly higher in both cover crop treatments than in CLT, with WC treatment (3498 g N ha^{-1}) and WCR treatment (4149 g N ha^{-1}) exceeding CLT (1064 g N ha^{-1}) by 69% and 74%, respectively.

Average total N loss from N-fertilized treatments through runoff accounted for 3.7%–5.5% of total urea-N applied over 2 years of observation, which was in the lower range of 3%–10% reported by others in pasture field studies (Kleinman et al., 2006). One possible explanation that affected the runoff intensity was the smaller overall ground slope (5%) in this study site compared to 11%–13% from Kleinman et al. (2006). Additionally, vegetation coverage is another factor that could affect the runoff volume (Kleinman et al., 2006; Udeigwe et al., 2010). The runoff N loss was less during the first fertilization period when cover crops were alive than the second fertilization period. Overall, treatments with winter cover crops showed a decrease in runoff volume of up to 50% in this study (Table S3). This suggested that clover cover crop could reduce the overall runoff N loss through the mulch effect when used to substitute a portion of N fertilizer.

3.5 | N_2O emission from WC residue-amended soil with N stabilizers

To reduce N_2O emission from clover residue degradation of bio-fixed N source, a laboratory incubation of soil–clover biomass mixtures treated with N stabilizers was carried out for 20 days, and the results are shown in Figure 5. N_2O emissions from WC-amended soil samples were affected by N stabilizer type and application rate. The incorporation of WC matter into soil emitted a significant amount of N_2O, with the flux peaks appearing immediately after the soil being wetted. The N_2O flux then gradually dropped as the soil dried, which agreed with the N_2O flux pattern from the field experiment. The 100% DCD application rate significantly reduced N_2O emissions compared to the 10% application rate. The 10% DMPP treatment was more effective at mitigating N_2O emissions than the 10% DCD treatment, and NBPT showed no significant reduction compared to the clover CTL without inhibitors (Figure 5). Overall, compared to the cumulative N_2O emission in clover (1.88 mg N) in the 20-day incubation, the emissions were significantly reduced by DCD 100% (0.17 mg N, −91%), DCD 10% (1.02 mg N, −46%), and DMPP 10% (0.43 mg N, −77%), while NBPT (1.75 mg N) showed no significant difference.

The 46% reduction in N_2O emission from decomposing clover-fixed N with DCD 10% as observed in this study was lower than the 76% N_2O reduction from urea-N using the same rate of DCD reported in our previous study (Meng et al., 2021). This indicates a limited mitigation efficiency of N_2O emission from bio-fixed N decomposition of legume biomass. On the other hand, this study showed a greater N_2O reduction with DMPP 10% treatment, up to 77% efficiency, indicating that DMPP might be more effective at treating clover-N at an equivalent concentration to DCD. Weiske et al. (2001) reported that DMPP had a similar reduction of N_2O emissions as DCD at one-tenth the rate of DCD. While this result strongly suggests that commonly used nitrification inhibitors such as DCD and DMPP could be used for minimizing N_2O emission from the degradation of legume cover crops, the specific rates need to be determined from further field studies under different climate conditions. On the other hand, NBPT, a urease inhibitor, showed no improvement in controlling N_2O emission induced by WC decomposition. Although NBPT was observed to have a significant effect on reducing N_2O emission from urea- and urine-fertilized pastures (Ding et al., 2015; Krol et al., 2020; Singh et al., 2013; Zaman et al., 2008), it had little influence on the mineralization of bio-fixed N, where the major N forms are protein and amino acids.

4 | CONCLUSIONS

This study demonstrated that WC as a cover crop, combined with a 50% N application rate, can reduce N fertilizer input while maintaining bermudagrass yields comparable to a full N application rate. The clover-substitute plots also had less runoff N loss due to decreased runoff volume. However, the clover-substituted plots had similar amounts of N_2O emissions as those receiving full fertilizer (urea) N application over 2 years. The ryegrass–WC mixture increased bermudagrass N

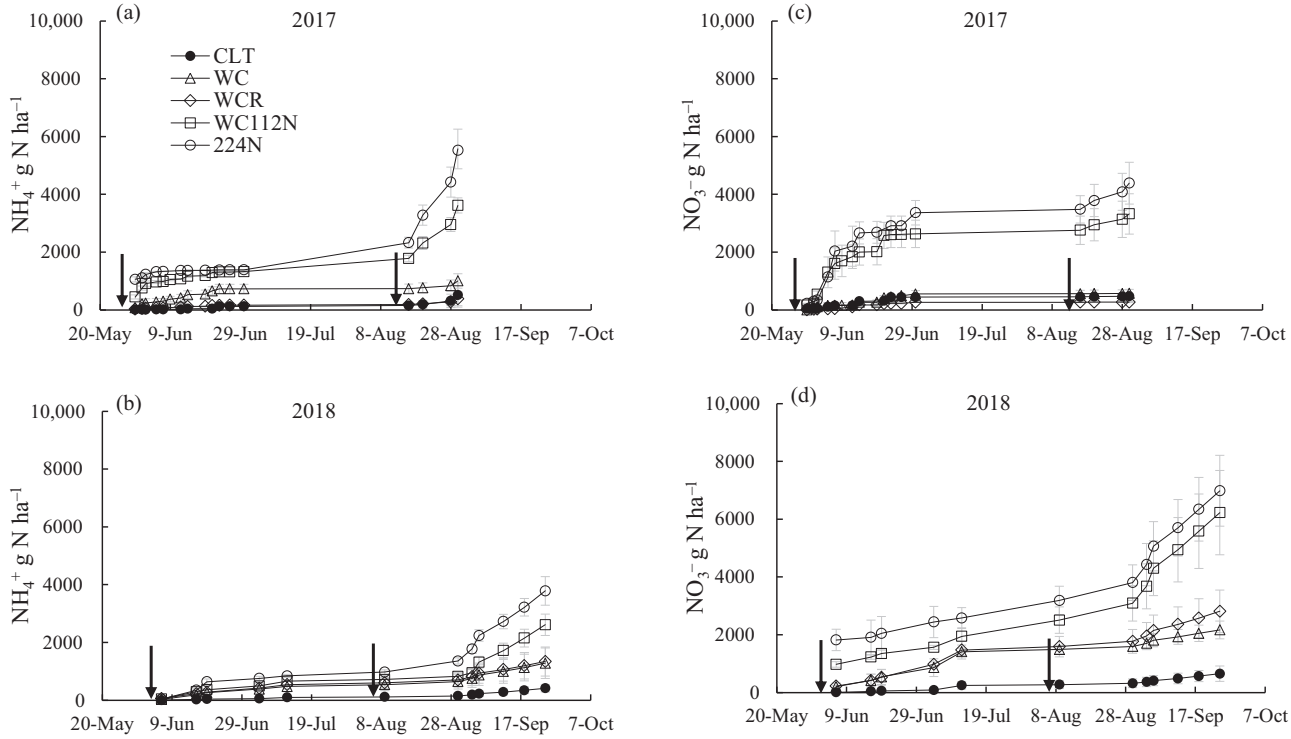


FIGURE 4 Cumulative load of NH_4^+ and NO_3^- in runoff water in 2017 (a and c) and 2018 (b and d). Bars indicate standard errors among replicates. Arrows indicate the date of N fertilization. 224N, 224 kg urea-N ha^{-1}; CLT: control; WC: white clover; WC112N: white clover with 112 kg urea-N ha^{-1}; WCR: white clover with ryegrass.

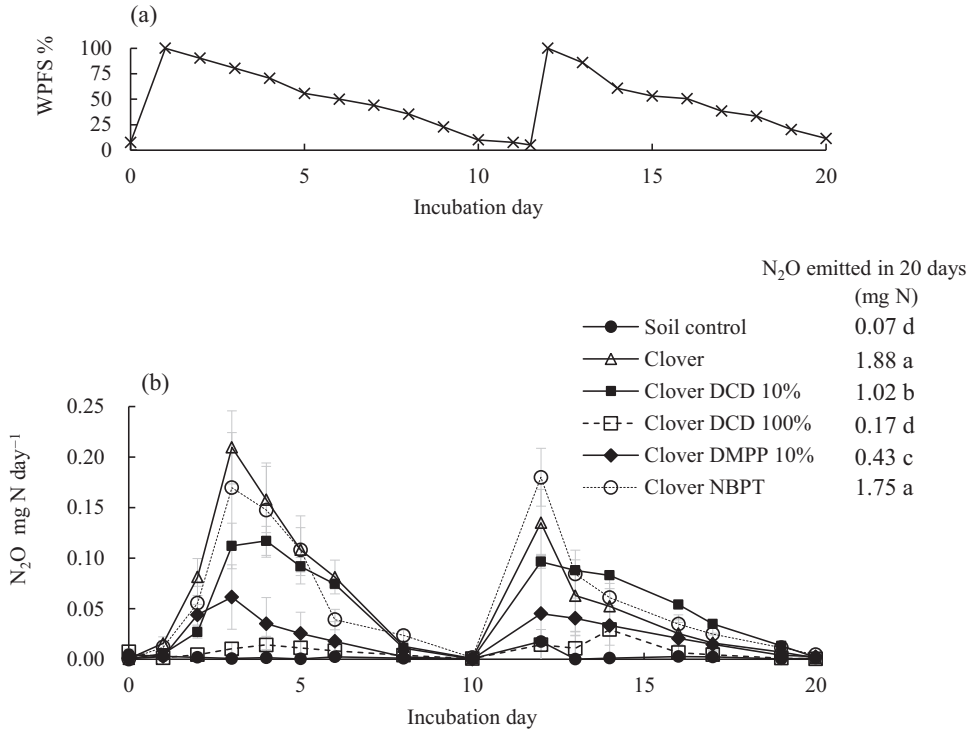


FIGURE 5 Water-filled pore space (a) and the temporal N_2O emission from white clover biomass amendment (b) in a 20-day soil incubation with N stabilizers. Bars indicate standard errors among replicates. Letters after numbers indicate significance among treatments using Tukey's least significant difference (LSD) comparison ($\alpha < 0.05$).

yield compared to no cover crop, although yields remained lower than pure WC stands over 2 years. The inclusion of ryegrass in the mixture showed no significant effect on water runoff N losses compared to pure WC stands. Use of nitrification inhibitors, DCD or DMPP, could significantly reduce N_2O emission from the mineralization of clover residue, while urease inhibitor NBPT had little effect in the incubation experiment. Overall, the use of WC as a winter cover crop in bermudagrass systems may reduce the need for mineral N fertilizer but with no reduction in cumulative N_2O emissions. Similar to mineral N fertilizers, nitrification inhibitors might be effective in lessening N_2O emissions from clover residues.

AUTHOR CONTRIBUTIONS

Yili Meng: Data curation; formal analysis; investigation; writing—original draft. **Jim J. Wang**: Conceptualization; funding acquisition; methodology; project administration; resources; supervision; writing—review and editing. **Syam K. Dodla**: Methodology; writing—review and editing. **Lewis A. Gaston**: Resources; writing—review and editing. **Zhuo Wei**: Data curation; resources. **Jiabing Li**: Resources. **Guillermo Scaglia**: Resources. **Jong-hwan Park**: Resources.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

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