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Glyphosate efficacy, absorption and translocation for *Eragrostis* plana control

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Abstract: **Background**: Glyphosate is the most effective herbicide to control the invasive and perennial *Eragrostis plana* in Southern Brazil. However, one application has not been sufficient to prevent the regrowth of plants.

Objective: The aim of this research was to evaluate the efficacy of glyphosate sprayed alone or mixed with fluazifop-p-butyl or flumioxazin on *E. plana* control and to investigate ^{14}C-glyphosate absorption and translocation.

Methods: Plants with 2-3 tillers were treated with glyphosate (700 g a.e. ha^{-1}), fluazifop-p-butyl (47 g a.i. ha^{-1}), flumioxazin (100 g a.i. ha^{-1}), glyphosate + fluazifop-p-butyl (700 g a.e. ha^{-1} + 47g a.i. ha^{-1}), glyphosate + flumioxazin (700 g a.e. ha^{-1} +100 g a.i. ha^{-1}). Weed control was evaluated visually for 28 d after treatment (DAT) when plants were harvested for shoot dry biomass determination. ^{14}C-glyphosate absorption and translocation were measured at 24 and 72 h after treatment.

Results: Glyphosate at 700 g a.e. ha^{-1} controlled *E. plana* plants at 2-3 tillers. The mixture with fluazifop-p-butyl or flumizin did not significantly increase the level of control (87 and 86% at 7 DAT, respectively) but increased ^{14}C-glyphosate translocation to the roots.

Conclusions: Glyphosate is effective on *E. plana*. However, applied with fluazifop-p-butyl could improve the control of this weed, especially when perennial plants of *E. plana* dominate the field, preventing their fast regrowth.

Keywords: ^{14}C-glyphosate; fluazifop-p-butyl; forage grass; invasive plant; tough lovegrass

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1. Introduction

The structure and floristic richness of Southern fields of Brazilian rangelands in Rio Grande do Sul, Santa Catarina, and Paraná states are unique. Such diversity derives from a multitude of factors like climate, soil, and vegetation management (Boldrini, 2009). More than 450 species belonging to the family Poaceae and more than 200 species belonging to the family Fabaceae are distributed in this region and are economically relevant, given their forage profile (Boldrini, 2009). Despite the importance of and benefits from fields composing the "Pampa biome", they have been degraded due to several factors, among them, intense grazing, burning (although forbidden), mechanized cultivation practices (Medeiros, Focht, 2007), and biological invasion of exotic species (Pivello et al., 1999) such as tough lovegrass (*Eragrostis plana* Nees). This species has caused a severe biodiversity loss in the region, which is of great environmental concern (Medeiros, Focht, 2007).

E. plana was introduced into Brazil in the 1950s as a putative high-quality forage, but the low digestibility and poor nutrient composition did not allow its use as expected (Kissmann, Groth, 1999). *E. plana* invasion in native rangelands has contributed to the declining abundance of native grasses, affecting pasture quality and causing animal losses (Medeiros et al., 2009), including teething wear of livestock due to its very fibrous leaves. Recently, it was observed that *E. plana* and *E. pilosa,* another invasive species from Africa, could compete with irrigated rice crop (Carloto et al., 2019). High allelopathic potential has been observed in field and laboratory studies and is known to facilitate *E. plana* establishment and displacement of native flora. A study evaluating leaf extracts from *E. plana* on clover seed germination confirmed its allelopathic effect (Scheffer-Basso et al., 2019).

The best application timing for systemic herbicides, especially for the management of perennial weeds, corresponds to the stage where maximum basipetal translocation of carbohydrates occurs (Banks et al., 1977). This change in the translocation pattern of photoassimilates usually takes place after flowering and allows phloem-mobile herbicides, such as glyphosate, to reach roots and rhizomes, optimizing herbicide activity. Glyphosate is considered the best herbicide for *E. plana* management (Goulart et al., 2012; Bastiani et al., 2021). During the *E. plana* life cycle, the full-tillering stage is when plants are least sensitive to glyphosate (Bastiani et al., 2021), the cause of reduced glyphosate translocation into different plant parts, especially in older tillers. Instead, minimum amounts are distributed across some tillers, which results in sublethal concentrations on a per-tiller basis, allowing several tillers to regrow. *E. plana* does not have rhizomes. Nevertheless, Favaretto et al. (2015) found starch accumulation in roots of *E. plana*, which can explain the fast regrowth observed in the field after unsuccessful glyphosate applications. Incomplete control of perennial grasses with herbicides is a common problem.

The mixture of active ingredients can be beneficial to slow the evolution of herbicide resistance and increase the spectrum of controlled weeds (Norris et al., 2001). A herbicide mixture is synergistic when the effect is greater than the sum of both herbicides applied alone. However, if the observed effect of the mixture is lower than the sum of individual effects, it is classified as antagonistic (Colby, 1967). The interaction is additive if the sum of isolated effects does not differ from the mixture effect (Staker, Oliver, 1998). An interesting example is the synergistic interaction between glyphosate and clethodim or haloxyfop described for *Digitaria insularis* (L.) Fedde. control (Barroso et al., 2014). Herbicide options for *E. plana* management are limited, and, in general, are not as efficient as glyphosate when applied by themselves (Goulart et al., 2012; Faleiro et al., 2021). Therefore, farmers mostly rely on glyphosate mixtures to manage *E. plana* infestations. A commonly used partner for glyphosate - especially in soybean preplant burndown applications - is flumioxazin, a protoporphyrinogen oxidase (PROTOX) inhibitor. To a lower extent, glyphosate + fluazifop-p-butyl have also been used by farmers in Southern Brazil.

Similar to the *E. plana* case, several invasive plants worldwide have escaped cultivation or been introduced without a previous thorough evaluation (Nentwig et al., 2018). As a consequence, negative environmental impacts on native flora and fauna could occur if the species is not managed. Limited knowledge exists about herbicide behavior in *E. plana* plants during their life cycle, and additional research is needed to establish a correct management strategy. Knowledge about the absorption and translocation of glyphosate on *E. plana* is needed. Interestingly, Bastiani et al. (2021) found that the addition of ammonium sulfate to glyphosate increased translocation out of the treated leaf in *E. plana*. However, when associated with other herbicides, no information about glyphosate translocation is reported to manage this invasive plant.

Therefore, the objectives of this research were to: 1) evaluate glyphosate efficacy at 2-3 tiller stage when applied alone or in association with flumioxazin or fluazifop-p-butyl-p-butyl; and 2) evaluate the effect of these mixtures in glyphosate absorption and translocation.

2. Material and Methods

The experiments were carried out between December 2018 and January 2019 in a greenhouse and the Radioisotope Laboratory of Crop, Soil and Environmental Sciences Department da University of Arkansas (CSES/UARK), Fayetteville - AR, United States. Seeds of *E. plana* were collected in native pastures of the Federal University of Pelotas, Capão do Leão, RS, Brazil, in March 2018.

2.1 Glyphosate Efficacy

Experimental units were pots of 500 mL of field soil from the Arkansas Main Research Station at Fayetteville, CSES/UARK, United States. Seeds were sown, and seedlings were thinned to 1 plant per pot five days after emergence. *E. plana* plants were grown in a greenhouse maintained at 32/28 °C day/night temperature, with a 14 h light supplementation. When plants reached the 2- to 3-tiller stage, they were sprayed with one of the following herbicide treatments: glyphosate (700 g a.e. ha^{-1}), fluazifop-p-butyl-p-butyl (47 g a.i. ha^{-1}), flumioxazin (100 g a.i. ha^{-1}), glyphosate + fluazifop-p-butyl-p-butyl (700 g a.e. ha^{-1} + 47 g a.i. ha^{-1}) and glyphosate + flumioxazin (700 g a.e. ha^{-1} + 100 g a.i. ha^{-1}). An untreated check was included. The experiment consisted of a completely randomized design, with four replicates (one replicate = 1 plant per pot). Two runs of the experiment were conducted simultaneously in two different greenhouses. Herbicides were applied at a constant pressure of 280 kPa in a spray chamber equipped with a 1-m spray boom with two flat-fan nozzles (Teejet TP 800067), spaced 0.5 m apart, and calibrated to deliver a spray volume of 187 L ha^{-1}.

2.2 Absorption and translocation of glyphosate studies

E. plana plants were cultivated as previously described. At the 2- to 3-tiller stage, plants were sprayed with one of the herbicide treatments: glyphosate (700 g a.e. ha^{-1}), glyphosate + fluazifop-p-butyl (700 g a.e. ha^{-1} + 47 g a.i. ha^{-1}) and glyphosate + flumioxazin (700 g a.e. ha^{-1} + 100 g a.i. ha^{-1}). The same application equipment and parameters were used. Immediately after herbicide application, plants were taken to the radioisotope laboratory, where ^{14}C-glyphosate was applied. The radiolabeled herbicide was applied to the third youngest, fully expanded leaves using a microsyringe in four 1-μL droplets resulting in a total of 150,000 dpm of [^{14}C] glyphosate applied per plant. Plants were harvested at 24 and 72 hours after treatment (HAT).

To recover the unabsorbed glyphosate, treated leaves were harvested and triple-rinsed in 10 mL of a 9:1 mixture of water and methanol. A composite sample was made by mixing 5 mL aliquots of each replicate, and 10mL of scintillation cocktail was added (Ultima GoldTM; PerkinElmer Inc., Waltham, MA, USA). Radiation was quantified using a liquid scintillation spectrometer (LSS, Packard Tri-Carb 2100TR; Packard Instrument Corp., Downers Grove, IL, USA). Glyphosate absorption was calculated as the percentage of total radioactivity recovered (rinsate + radioactivity the plant) as described by the following equation:

Absorption (%) = Total of radioactivity in tissues / Total of Radioactivity in tissues + radioactivity from leaf rinsate x 100

Glyphosate translocation was evaluated by dividing the treated plant into sections: treated leaf (TL), above TL, below TL, tillers, and roots, for each harvest time, which were dried at 60°C for 48 h. After that, they were oxidized in a biological oxidizer (OX500TM; R. J. Harvey Instrument Corp., Tappan, NY, USA). The $^{14}CO_2$ evolved during sample combustion was trapped in a glass scintillation vial containing 15 mL of scintillation cocktail (Carbon-14 Cocktail; R. J. Harvey Instrument Corp.). It was quantified using the LSS, as previously described.

One plant from each harvest period and treatment was used to visualize herbicide translocation through autoradiographs. Plants were collected at 24 and 72 HAT and immediately pressed in absorbent paper. Leaves were placed to avoid the treated leaf contacting other parts of the plant. Plants were dried and, subsequently, exposed to a phosphorescent film for 48 h. After which they were scanned in Storm 820 PhosphorImagerTM (Molecular Dynamics Inc., Sunnyvale, CA, USA).

2.3 Statistical analysis

Herbicide effect was visually assessed using a 0 to 100% scale at four intervals: 7, 14, 21, and 28 days after treatment (DAT), where 0% represents the absence of symptoms, and 100% represents plant death (Sociedade Brasileira da Ciência de Plantas Daninhas, 1995). After the last visual assessment, shoots were harvested and dried at 60 °C to determine the final shoot dry mass. Data was checked for normality (Shapiro-Wilk), and an analysis of variance was performed considering run as fixed effect using the function lmer in the package lme4 (version 1.1-26), in R 4.0.3 (R Core Team, 2019). Because no significant effect was observed ($p \geq 0.05$), data from both runs of the experiments were combined, and means were compared using the Tukey's test (package ExpDes1.2.1). Statistical analyses were performed using R software (R Core Team, 2019).

3. Results and Discussion

E. plana control with glyphosate reached 95% at 7 DAT (Table 1), and 100%at 14 DAT. Glyphosate + fluazifop-p-butyl or flumioxazin provided control of 87% and 86% at 7 DAT, respectively, with means not differing from glyphosate applied alone. Fluazifop-p-butyl and flumioxazin applied by themselves were not different from each other. Only 32 and 30% control of *E. plan*a at 7 DAT were observed for fluazifop-p-butyl and flumioxazin respectively. However, at 14 DAT, fluazifop-p-butyl provided better control than flumioxazin, 76% and 32%, respectively. The greatest control achieved with fluazifop-p-butyl by itself was observed at 28 DAT (88%), at which time flumioxazin provided 39% control of *E. plana* (Table 1). Glyphosate was efficient in controlling young plants of *E. plana* (2- to 3-tiller stage), which was not surprising considering that young plants have less biomass and higher herbicide absorption than old plants (Degreeff et al., 2018). In addition, the translocation patterns of photoassimilates can change considerably during the plant is life cycle, affecting herbicide activity (Fadin et al., 2018).

In general, the addition of other herbicides does not affect the efficacy of glyphosate when it is applied at a stage when the weed is highly susceptible; however, glyphosate efficacy can be compromised by certain herbicide mixtures when applied at a suboptimal growth stage or during suboptimal environmental conditions. To illustrate this point, the addition of 2.4-D, chlorimuron, metolachlor, or cloransulam to glyphosate did not affect *Spermacoce verticillata* control, when plants were sprayed at the 2- to 4-leaf stage. At that stage, glyphosate alone or in combination provided high levels of control. However, when plants were sprayed at the 4- to 6-leaf stage, the glyphosate + cloransulam mixture was 40% more effective compared to glyphosate applied alone (Fadin et al., 2018). Likewise, glyphosate alone or in combination with quizalofop or clethodim was equally effective in controlling *D. insularis* sprayed at the 4-leaf stage. However, these herbicides were synergistic when applied to bigger plants (3 to 4 tillers) (Barroso et al., 2014). While glyphosate alone provided 48% control, the addition of quizalofop or clethodim resulted in 79% and 81% control, respectively (Barroso et al., 2014).

The glyphosate + flumioxazin treatment was included in our study as it is commonly used by farmers in Southern Brazil, before to soybean cultivation in crop-livestock integration system areas. Farmers reported improved *E. plana* control in the field (personal communication), but we did not observe this in our test where glyphosate alone already provided highest control (>90%). A repetition of this study using older plants beyond four tillers, could be informative. As flumioxazin had very poor activity on *E. plana* control (< 39% at 28 DAT) (Table 1), we believe that its use by farmers mixed with glyphosate is justified by its residual soil activity (Oliveira et al., 1998; Carvalho, 2017), which would reduce the establishment of *E. plana* seedlings from the seed bank. This assumption needs further investigation. The mixture of glyphosate with other herbicides can have different results (Staker, Oliver, 1998). This research detected no visible improvement in glyphosate efficacy when mixed with fluazifop-p-butyl or flumioxazin herbicides.

The total amount of glyphosate absorbed by *E. plana* at 24 HAT reached 25% of the applied, 41% for glyphosate + fluazifop-p-butyl, and 28% for glyphosate + flumioxazin (Figure 1A). Hence, the addition of fluazifop-p-butyl to glyphosate favored its early absorption. Interestingly, flumioxazin enhanced glyphosate absorption at 72 HAT but limited its translocation: 60% of the absorbed herbicide remained in the treated leaf (TL) (Figure 1B). PROTOX-inhibitors have differential absorption by weeds, but in general, their translocation is limited (Vanstone, Stobbe, 1978; Ritter, Coble, 1981; Higgins et al., 1998; Unland et al., 1999). Glyphosate isolated showed less total absorption and translocation than when mixed with fluazifop-p-butyl or flumioxazin. However, it was not observed difference on *E. plana* control (Table 1). Possibly, a synergistic effect explain this and need further investigation based on studies of the mixture of glyphosate with other herbicides (Staker, Oliver, 1998; Barroso et al., 2014).

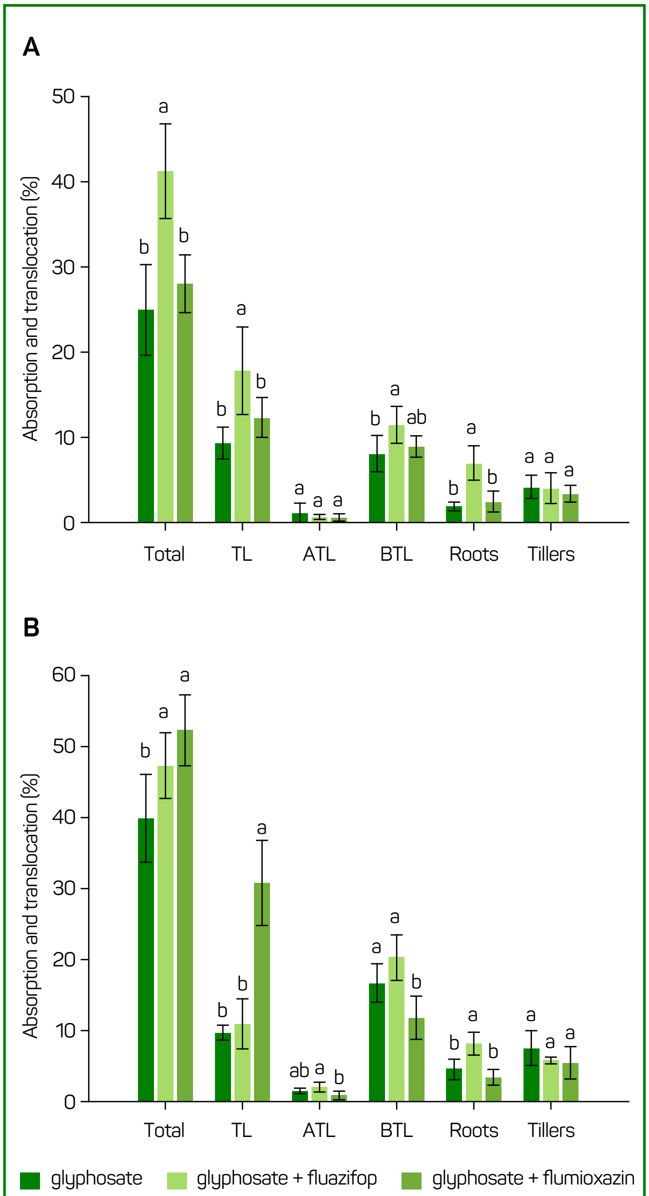

Figure 1 - Percentage of absorption and translocation of ^{14}C-glyphosate at 24 (A) and 72 (B) hours after treatment: Total (absorption and translocation), treated leaf (TL), above treated leaf (ATL), below treated leaf (BTL), roots and plant tillers of *Eragrostis plana*. Herbicides applied at 2-3 tillers stage. Each plant received a total of 150,000 dpm of [^{14}C] glyphosate. Different lowercase letters indicate statistical differences among plant parts by Tukey's test ($P \leq 0.05$). Bars represent the standard error. Fayetteville/AR, USA, 2018/19.

Perhaps the most interesting result of this research was that the addition of fluazifop-p-butyl to glyphosate increased glyphosate movement to the roots regardless of the evaluation timings. This was clearly seen in the autoradiographies. As mentioned before, we were not able to observe additive or synergistic effects because glyphosate alone provided excellent control of *E. plana*. However, the improved translocation to the roots indicates the mechanism through which fluazifop-p-butyl may enhance *E. plana* control under field conditions. Glyphosatetranslocation in plants goes in both directions, the root system and shoot, and follows the flow of carbohydrates from sources towards sinks (amphimobile). The herbicide has a tendency to accumulate in the apical meristems of leaves and roots (Chen et al., 2009; Reis et al., 2015). Some *E. plana* features, such as high lignin concentration in leaves (Favaretto et al., 2015), and the possible interdependence mechanism between early tillers (Corrêa et al., 2014; Bastiani et al., 2021), tend to impair glyphosate translocation. The associated use of glyphosate with other herbicides capable of improving its translocation is an option to be considered for managing perennial species such as *E. plana*. However, further studies to assess the translocation of ^{14}C-glyphosate/fluazifop-p-butyl at different invasive plant development stages are needed.

The knowledge generated in this research can be used to optimize a technique for *E. plana* control that is currently used in Southern Brazil. The equipment called *Campo Limpo*, developed in collaboration with Brazilian Agricultural Research Corporation (*Empresa Brasileira de Pesquisa Agropecuária* – Embrapa), selectively applies glyphosate on the target plants without exposing the desired forage to the herbicide (Perez, 2015). However, weed control is many times unsatisfactory given the fact that plants are distributed in dense tussocks, which reduce herbicide translocation throughout the whole plant and lead to resprouting. In this sense, the addition of fluazifop-p-butyl to glyphosate may enhance *Campo Limpo* efficiency and practicality.

4. Conclusions

Glyphosate at 700 g a.e. ha^{-1} effectively controlled *Eragrostis plana* when applied at the 2- to 3-tiller stage.

Table 1 - Control (%) of *Eragrostis plana* at 7, 14, 21, and 28 days after application of the herbicide treatment (DAT) and shoot dry biomass (SDB) reduction at 28 DAT. UARK, Fayetteville/AR, USA, 2018/19.

Treatments	Dose (g e./i. a ha^{-1})	Control (%)				SDM (%)
		7 DAT	14 DAT	21 DAT	28 DAT	28 DAT
glyphosate	700	95 a*	100 a	100 a	100 a	95 a
fluazifop-p-butyl	47	32 b	52 b	76 b	88 b	93 a
flumioxazin	100	30 b	30 c	32 c	39 c	55 b
glyphosate+fluazifop-p-butyl	700+47	87 a	97 a	100 a	100 a	96 a
glyphosate+flumioxazin	700+100	86 a	93 a	96 a	98 a	96 a
Untreated check	---	0 c	0 d	0 d	0 d	0 c
C.V. (%)**		9.61	3.57	3.08	2.20	15.09

*Means followed by the same letters indicate no statistical differences among treatments by Tukey´s test ($P \leq 0.05$).

**Coefficient of variation.

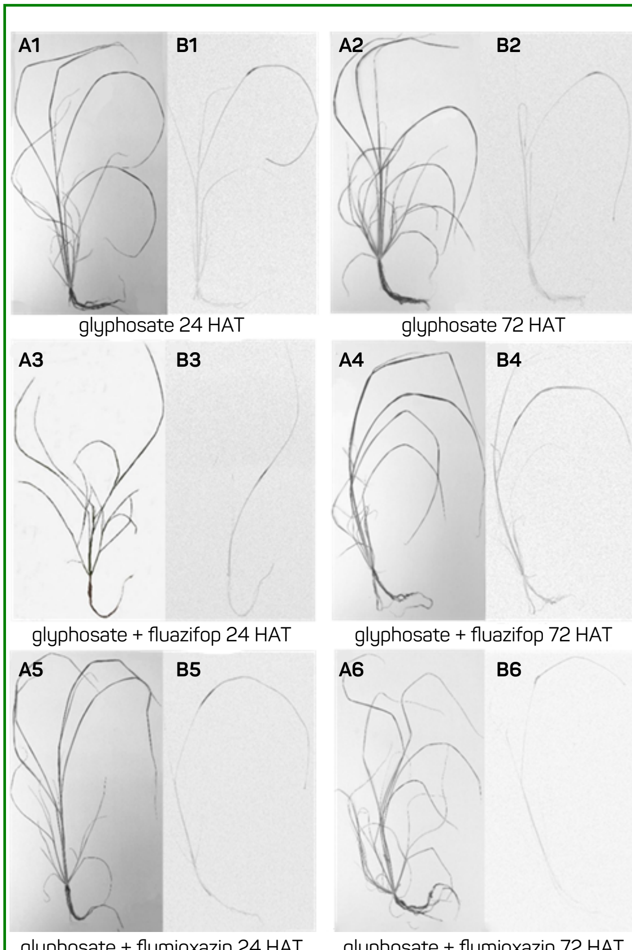

Figure 2 - Autoradiograph of *Eragrotis plana* with glyphosate (700 g e.a ha^{-1}), glyphosate + fluazifop-p-butyl (700 g e.a + 47 g i.a ha^{-1}) and glyphosate + flumioxazin (700 g e.a + 100 g i.a ha^{-1}) added with ^{14}C-glyphosate at 24 and 72 HAT. (A) pressed plant (B) translocation autoradiograph. Fayetteville/AR, USA, 2018/19.

The addition of fluazifop-p-butyl or flumioxazin to glyphosate does not improve *E. plana* control. However, the mixture with fluazifop-p-butyl increased ^{14}C-glyphosate translocation in the invasive plant and may be useful for managing adult plants in highly infested fields.

Author's contributions

All authors read and agreed to the published version of the manuscript. FCC, FPL, and NR: conceptualization of the manuscript and development of the methodology. FCC, MLZ, and MNG: data collection and curation. FCC, MNG, and MLZ: data analysis. FCC, and MNG: data interpretation. NR, and FPL: funding acquisition and resources. FPL, and NB: project administration. FPL, NR, and LAA: supervision. FCC: writing the original draft of the manuscript. FCC, FPL, MNG, MLZ, NR, and LAA: writing, review and editing.

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