## **UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ**

## **Colegio de Posgrados**

## Evaluating the impact of horse trampling on Carbon Dioxide and Methane emissions on a high-elevation páramo peatland in the Northern Andes of Ecuador

Tesis

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## **COLEGIO DE POSGRADOS**

# HOJA DE APROBACIÓN DE TRABAJO DE TITULACIÓN

## Evaluating the impact of horse trampling on Carbon Dioxide and Methane emissions on a high-elevation páramo peatland in the Northern Andes of Ecuador

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### **RESUMEN**

Las turberas andinas de alta montaña han sido drenadas y utilizadas para el pastoreo durante al menos 200 años, liberando grandes cantidades de carbono a la atmósfera. Restaurar estas turberas y evaluar cómo la dinámica del carbono se ve afectada por la actividad humana es un paso crucial para aumentar el sumidero de carbono de la tierra y contribuir a alcanzar los objetivos de emisiones netas cero. El objetivo de este estudio es caracterizar la dinámica del carbono de una turbera restaurada y evaluar cómo afecta a este proceso el pisoteo por caballos. Se realizó un experimento de campo para comparar los flujos de carbono entre un gradiente de intensidad de pisoteo. Para las mediciones previas al pisoteo, el intercambio neto de carbono en el ecosistema (NEE) alcanza -0,65 g CO2/m2/hora, lo que significa un secuestro de carbono. En las mediciones posteriores al pisoteo, incluso a bajas intensidades de pisoteo, obtuvimos un cambio de sumidero de carbono a fuente de carbono y el impacto es mayor a intensidades más altas, con una reducción del 102% al 136%. Además, las emisiones de metano aumentan entre 3 y 10 veces en comparación con nuestras parcelas de control y este gran aumento está estrechamente relacionado con el pisoteo de los caballos. Nuestros hallazgos representan una fuerte evidencia a favor de priorizar el manejo adecuado de las turberas altoandinas como herramienta para mitigar el cambio climático.

Palabras clave: turberas andinas, andes del norte, pisoteo de caballos, dinámica de carbono, dióxido de carbono, metano.

#### ABSTRACT

Andean high mountain peatlands have been drained and used for grazing for at least 200 years, releasing large amounts of Carbon into the atmosphere. Restoring these peatlands and assessing how carbon dynamics are affected by human activity is a crucial step to increase the land's carbon sink and contribute to achieving net zero goals. The purpose of this study is to characterize the carbon dynamics of a restored peatland and assess how trampling by horses affects this process. A field experiment was executed to compare carbon fluxes between a trampling intensity gradient. For pre-trampling measurements, the net carbon ecosystem exchange (NEE) reaches -0.65 g CO<sub>2</sub>/m<sup>2</sup>/hour, which means a carbon sequestration. For post-trampling measurements, even at low trampling intensities we obtained a switch from a carbon sink to a carbon source and the impact is greater at higher intensities with a reduction from 102% to 136%. Additionally, the methane releases increase between 3 and 10 times compared to our control plots and this large increase is closely related to the horse trampling. Our findings represent strong evidence in favor of prioritizing adequate management for the Andean high mountain peatlands as a tool to mitigate climate change.

Keywords: andean peatlands, northern andes, horse trampling, carbon dynamics, carbon dioxide, methane.

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#### **INTRODUCTION**

Peatland ecosystems have been recognized as one of the largest reservoirs of carbon on Earth. At a global level peatlands store approximately one-third of the global soil carbon, while

4 covering only 3% of the worldwide surface (Urbina & Benavides, 2015; Xu et al., 2018).

5 Although most of this carbon is stored in northern temperate, and lowland tropical peatlands,

6 recent decades have brought attention to a vast systems of mountain peatlands that tend to

7 have a disproportionate role in biodiversity conservation and provision of ecosystem services.

8 A prominent example of these mountain peatlands is found in the páramos of the Northern

9 Andes of Venezuela, Colombia, Ecuador, and Perú.

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11 As a result of a complex topography shaped by glacial and volcanic activity, flat or low-lying 12 areas of the páramo landscape tend to accumulate water, creating anaerobic conditions in the 13 soil. The lack of oxygen, coupled with cool temperatures and other environmental factors, 14 result in the accumulation of peat, formed by incompletely decomposed plant detritus 15 (Hribljan et al., 2016; Xu et al., 2018). This low decomposition rate of organic matter is 16 caused by the biogeochemical interaction between metabolic activity of bacteria and fungi, 17 and the physical movement of electron acceptors and nutrients between oxic and anoxic 18 phases generated by the water in the subsoil (Limpens et al., 2008; Yu, 2011). The lack of 19 information on high-mountain tropical peatlands has led to their misrepresentation and they 20 have not been properly quantified on peatland maps on a global scale (e.g. Xu et al., 2018). 21

22 In the case of Ecuador, the páramo peatlands cover less than 1% of the surface of the entire 23 country, but store up to 2123 Mg/ha which could represent approximately 23% of the entire 24 carbon stock for all Ecuadorian forest biomass (Hribljan et al., 2017). Moreover, this estimate 25 might be an underestimation because of the methodological difficulties of sampling in remote 26 areas with complex mountainous topography, and very deep peat layers (Comas et al., 2017). 27 Despite their importance, páramo peatlands in Ecuador are threatened by anthropogenic 28 activities such as agriculture and grazing, which often requires the construction of drainage 29 ditches to remove the groundwater from the subsoil. Subsequently, the native vegetation is 30 replaced by more palatable introduced pastures species (Suarez et al., 2022). As a result of 31 these activities, lowered water tables and trampling can result in increased decomposition of

1 organic matter, and altered local hydrology which will affect carbon balance in the peatland.

2 Moreover, climate change is altering the hydrological conditions of high mountain peatlands

3 (Planas-Clarke et al., 2020), with poorly-understood consequences for the structure and

4 functioning of the ecosystem which haven't been properly understood.

5

6 Studies looking into the effects of land-use change on carbon dynamics of páramo peatlands 7 are scarce. Working in two peatlands in northern Ecuador, (Sánchez et al., 2017) showed that 8 methane emissions at a grazed peatland were approximately 17 times higher in comparison to 9 an undisturbed site. In the case of drained peatlands, it has been consistently reported that the 10 CO<sub>2</sub> production increases from respiration processes (Haddaway et al., 2014; Sánchez et al., 11 2017; Veber et al., 2018; Yuan et al., 2021). Previous studies have also reported changes in 12 physical properties of the soil that can lead to altered subsoil water conductivity and the water 13 table level, which ultimately affect carbon cycling (Limpens et al., 2008; Sánchez et al., 2017; 14 Urbina & Benavides, 2015).

15

16 Another common impact on mountain peatlands is trampling by native or introduced animals. 17 In the central and southern Andes, for example, South-American camelids (llamas, vicuñas 18 and guanacos) are commonly associated with mountain bofedales and vegas, while feral 19 horses and cows are frequent in páramo peatlands in Colombia and Ecuador. (Urbina & 20 Benavides, 2015) determined that rates of decomposition of aboveground organic matter 21 tripled in peatlands that experimented external fertilization and simulated trampling physical 22 disturbance. Similarly, trampling by horses in peatlands used for tourism and cattle have been 23 shown to result in vegetation loss (Barros & Pickering, 2015; Coronel et al., 2004). Based on 24 these studies, it could be projected that trampling could affect ecosystem carbon dynamics 25 through two main processes. First by altering soil physical conditions (e.g., bulk density, 26 water conductivity) which could affect microbial activity and respiration. Also, the organic 27 matter decomposition will be altered changing the methane fluxes. Second, by affecting the 28 structure and composition of the vegetation and, as a result its capacity to fix additional 29 carbon. Although these processes are critical in peatland ecosystems, their susceptibility to 30 trampling has not been assessed in peatlands of the northern páramos.

- 1 In this context, the main objective of this study is to explore the impacts of three levels of
- 2 trampling intensity on the patterns of CO<sub>2</sub> and CH<sub>4</sub> emissions in a páramo peatland in
- 3 northern Andes of Ecuador.
- 4

#### **METHODS**

#### 2 **2.1. Study area**

3 This study was carried out in the Chakana peatland, located in the Chakana Reserve (3750 m) 4 owned by Jocotoco Foundation, in the buffer zone of the Antisana National Park. This 5 peatland is part of the historical Hacienda Antisana which was reputed as «the highest farm» 6 in Ecuador at the end of the 19th century (Whymper, 1891), where the drainage marks were 7 evident in satellite imagery. As part of the ancient cattle rearing activities in the area, the 8 Chakana peatland was drained to remove groundwater from the subsoil, through the carving 9 of eight drainage ditches. Additionally, in a more recent period (c.a. 2015), 20 additional 10 ditches were constructed to create habitat for migratory birds. The effects of the ditching and 11 cattle activity resulted in lowered water table and a complete replacement of the peatland 12 native vegetation with exotic pasture species. Starting in 2017, this site has been restored by 13 blocking ditches to reduce water flow and allow rewetting of the peatland. The restoration has 14 been highly effective, as evidenced by a significant increase of the ground cover by peatland 15 species; (e.g., Eleocharis dombeyana, Caltha sagitatta, Juncus arcticus) and a reduction in 16 the area of open water in the ditches (Suarez et al., 2022). Now Chakana is a peatland with 17 different levels of restoration showing a great opportunity to evaluate the impacts of common 18 anthropogenic activities and the impacts of restoration.

## 19 2.2. Experimental design

20 In order to evaluate the impacts of the trampling intensity, nine experimental blocks were

- 21 established. The blocks were spatially distributed in an attempt to cover the variability in
- 22 water table levels (WTL) across the peatland (Figure 1). Each block contained four 2 m radius
- 23 circular plots which were randomly assigned to one of four treatments: No trampling
- 24 (Control), and three levels of trampling intensity: high, medium, and low (Figure 2). At the
- 25 plots assigned to trampling treatments, vegetation was cut at ground level using a string grass
- 26 trimmer to simulate vegetation removal by feeding horses. This procedure was carried out
- 27 only once at the beginning of the experiment.
- 28 Trampling treatments were administered by walking an adult horse around each circular plot,
- 29 for a maximum of 3 minutes (Figure 3). The intensity of the treatments was regulated by
- 30 controlling the periodicity of the trampling. The high intensity plots were trampled once every

month while the medium and low intensity plots were trampled every two and three months,
 respectively.

3 We collected three rounds of baseline (pre-trampling) field measurements from April 2022

4 through June 2022. Implementation of trampling treatments started in July 2022, we

5 completed ten rounds of post-trampling measurements which were concluded in March 2023,

6 after three full cycles of trampling treatment. In addition, for the last two rounds of field

7 measurement, we designated additional control plots in five blocks, in which, unlike the

8 original control plots, their vegetation covers were also removed. As the patterns of NEE

9 could have been affected by the initial cut of the vegetation in the trampling plots, we

10 implemented an additional set of control plots in which the vegetation was cut, but no

11 trampling was implemented. These plots were sampled twice in January and March 2023, to

12 assess the potential influence of the removal of the vegetation.

### 13 **2.3.** Carbon dioxide and methane fluxes

14 To monitor the gas fluxes in situ a portable Picarro ® GHG analyzer with cavity ring-down spectroscopy was used. This instrument has a precision of 3 ppb for Methane and 0.4 ppm for 15 16 carbon dioxide (Picarro Inc., 2021). This device is connected to the soil through a PVC ring 17 that could vary in height depending on the height of the vegetation. The GHG analyzer uses 18 the method proposed by Hutchinson & Mosier (1981) to fit a curve in a two-minute 19 measurement that allows for transformation from concentration to gas flux. The 20 measurements of CO<sub>2</sub> and CH<sub>4</sub> fluxes were performed only during daylight hours and only 21 when no rain was present. At each sampling point, CO<sub>2</sub> and CH<sub>4</sub> measurement were first 22 performed with the chamber uncovered to allow photosynthesis. Changes in CO<sub>2</sub> 23 concentration in the chamber during this first measurement are used to estimate net ecosystem 24 exchange (NEE), with negative values representing net carbon loss during that measurement. 25 Following this measurement, the chamber was covered with an opaque cloth to block 26 photosynthesis, and CO<sub>2</sub> fluxes were measured again. The expected increase in CO<sub>2</sub> 27 concentration in the chamber represents ecosystem respiration (ER). Based on these two 28 measurements, GPP was calculated as the difference between NEE and ER. During each gas 29 measurement, additional environmental variables were sampled including solar radiation, 30 relative humidity, soil temperature, air temperature, soil water content, and barometric

31 pressure.

#### 1 **2.4. Water table levels**

To determine the relationship between the water table level and gas emissions, one well was installed in each experimental block. Which consists of PVC tube of 1,5 m long and 10 cm in diameter, the tube was buried in the soil with holes that allowed the water flow and with a cloth filter to avoid clogging. Water table levels were monitored manually once per month.

#### 6 2.5. Bulk density and soil carbon content

In the 36 measurement plots, soil samples were taken in 10 cm height, kopecky rings with one
replicate per each plot. After that, each sample was divided in two subsamples from 0 cm to 5
cm and from 5 cm to 10 cm. The samples were taken once in June 2021, before
implementation of trampling treatments, and once in March 2023, at the end of the field
experiment. Bulk density was determined by drying the samples at 65° C for at least 4 days
and weighing. After that, a portion of the sample was incinerated in a muffle furnace at 550°
during 4 hours for percent organic matter determination (Blake G. R., 1965).

#### 14 **2.6.** Data analysis

15 All the data was managed with R software to develop descriptive and analytical statistics.

16 Generalized Linear Models (GLM) were executed to analyze the influence of the trampling

17 intensity, WTL, and PAR in the response of the NEE, ER and CH<sub>4</sub>. Previously, the NEE, ER

18 and GPP were normalized through cubic root transformation, and for the PAR

19 (Photosynthetically Active Radiation) and WTL the Log+1 was executed. Additionally, to

20 evaluate the trampling influence, comparing the pre-trampling data with the post-trampling

21 data, an additional model was carried out where the trampling was assigned as a factor.

22 Specific differences among treatment levels were explored through a post-hoc analysis using

the Holm method which is an adjustment of the Bonferroni method and provides a better

24 statistical power while maintaining the level of significance (Abdi, 2010).

25 Finally, to analyze the differences between the pre and post trampling soil samples in their

26 bulk density and soil carbon content, paired t tests were executed, and the Cohen d index was

27 calculated to assess the magnitude of influence from the trampling among the differences in

the soil characteristics.

#### **3. RESULTS**

#### 2 **3.1. Carbon dioxide fluxes**

3 Base-line values of NEE, ER, and GPP were similar across all plots (Figure 4) with a mean

- 4 NEE value of  $-0.65 \pm 0.03$  g CO<sub>2</sub>/m<sup>2</sup>/hour. Mean ER was  $0.65 \pm 0.02$  g CO<sub>2</sub>/m<sup>2</sup>/hour and
- 5 mean GPP was  $-1.3 \pm 0.04$  g CO<sub>2</sub>/m<sup>2</sup>/hour. Positive values of NEE across the plots imply that

6 the system was functioning as a net sink of carbon.

- 7 After trampling treatments were administered, mean NEE of the trampled plots became
- 8 significantly different from the control plots (Table 1). While in the control plots the mean
- 9 NEE was  $-0.77 \pm 0.09$  g CO<sub>2</sub>/m<sup>2</sup>/hour (similar to base-line values), NEE decreased by 136%

10 at the high intensity plots ( $0.28 \pm 0.08$  g CO<sub>2</sub>/m<sup>2</sup>/hour; p < 0.001) and by 114% and 102% at

- 11 the medium and low intensity plots, respectively (medium intensity:  $0.11 \pm 0.10$  g
- 12  $CO_2/m^2$ /hour; low intensity:  $0.02 \pm 0.09$  g  $CO_2/m^2$ /hour). As expected, NEE was also
- 13 significantly affected by PAR levels (p < 0.01). No significant effects were found for WTL (p
- 14 < 0.1), and for the interaction between high intensity treatment and PAR (p < 0.05). The
- 15 combination of the GLM models explains round to 43% of the variation of NEE.
- 16 Mean ER in the control plots was  $0.83 \pm 0.05$  g CO<sub>2</sub>/m<sup>2</sup>/hour and no significant difference
- 17 was found between trampling intensities, with averages of  $0.74 \pm 0.05$  g CO<sub>2</sub>/m<sup>2</sup>/hour,  $0.78 \pm$
- 18 0.05 g CO<sub>2</sub>/m<sup>2</sup>/hour and 0.81  $\pm$  0.05 g CO<sub>2</sub>/m<sup>2</sup>/hour for the high, medium, and low intensity
- 19 plots respectively. ER was significantly related to mean WTL (p < 0.001). The overall GLM
- 20 models explains round to 34% of the variation of the ER.
- 21 To determine the influence of the trampling we compared the pre-trampling and post-
- trampling measurements. Only in the case of NEE, significant differences were obtained
- between pre-trampling and post-trampling phases (p < 0.001) for high, medium, and low
- intensity (p < 0.001). In this case, the overall GLM explains round to 39% of the variation of
- 25 the NEE. For the ER, significant differences were obtained for the trampling variable (p <
- 26 0.05) and for the high trampling intensity (p < 0.05) and the combination of GLM models
- 27 explains only round to 3% of the variation in the response variable.

- 1 Additionally, the post-hoc analysis (Table 2) reported that the control plots were significantly
- 2 different to the high, medium, and low intensity trampling plots (p < 0.01) and a significant
- 3 difference was determined only between the high and low intensity plots (p < 0.05).
- 4 Carbon dynamics at the additional control plots where vegetation was cut, but no trampling
- 5 occurred, was characterized by a rate of NEE of  $-0.48 \pm 0.31$  g CO<sub>2</sub>/m<sup>2</sup>/hour and an ER 1,48 ±
- 6 0.21 g  $CO_2/m^2$ /hour for the ER (Figure 5).

### 7 **3.2. Methane fluxes**

- 8 Pre-trampling methane emissions averaged  $3.37 \pm 0.19$  mg CH<sub>4</sub>/m<sup>2</sup>/hour (Figure 6) and were
- 9 not significantly different among treatments. After trampling, methane emissions at the
- 10 trampling treatments increased dramatically and were between 3 and 10 times higher than in
- 11 the control plots (high intensity:  $30.85 \pm 15.69$  mg CH<sub>4</sub>/m<sup>2</sup>/hour (p < 0.001); medium
- 12 intensity:  $9.71 \pm 2.18 \text{ mg CH}_4/\text{m}^2/\text{hour } (p < 0.05)$ ; low intensity:  $25.73 \pm 17.25 \text{ mg}$
- 13  $CH_4/m^2/hour (p < 0.01)$ ). Methane emissions were also significantly related to WTL level (p
- 14 < 0.001). For methane measurements the combination of the GLM explains 29.33% of the
- 15 variation.
- 16 Post-hoc analysis (Table 3) showed that the control plots were significantly different from the
- 17 high, medium, and low intensity plots (p < 0.01). For methane emissions, the fluxes across
- 18 time are shown in Figure 7 and are expressed as a logarithmic form of the methane emission
- 19 account for the high dispersion of the data.

### 20 **3.3. Bulk density and soil carbon content**

- For the soil carbon content analysis (Table 4), no significant differences were found betweentreatments.
- 23 On average, bulk density tended to decrease between the pre-trampling and post-trampling
- sampling periods. However, the most noticeable pattern was an increase in the variability in
- 25 the post-trampling values of bulk density (Figures 8 and 9). Significant differences in bulk
- 26 density were found between high trampling intensity plots and control plots for the 5 to 10 cm
- 27 section of the subsoil.

#### 4. DISCUSSION

20

2 Trampling treatments in this field experiment resulted in a significant change on carbon 3 dynamics. In the case of CO<sub>2</sub>, pre-trampling values of NEE were negative (Figure 4) 4 suggesting that the peatland was acting as a net sink of carbon. After trampling, NEE values 5 became positive, while remaining negative at the control plots. Hence, or results suggest that 6 trampling by the horses turned our experimental plots into net CO<sub>2</sub> sources. Interestingly, this 7 change from carbon sink to source occurred at all trampling intensities, implying that peatland 8 soils can be vulnerable to the impacts of this activity, even at low levels of intensity. 9 In terms of the processes, our data suggest that the observed reduction in carbon sequestration 10 at our experimental plots (positive NEE), might be a direct result of the reduction in GPP,

resulting from the impacts of trampling on the vegetation. This conclusion is supported by the lack of significant differences in ER across post-trampling treatments, which -suggest thatsoil respiration and autotrophic respiration were not significantly altered by trampling. From this perspective, our results are consistent with another study in a trampled páramo peatland which showed that vegetation cover was a significant predictor of carbon dynamics, with

16 patches of low vegetation cover exhibiting positive values of NEE (Sánchez et al., 2017).

17 It must be noted that the reduction in GPP that we reported in the trampling treatments could 18 also be partially related to the initial clipping of the vegetation that we implemented in the 19 trampling plots. However, measurements in the additional plots in which the plants were 20 clipped but no trampling took place showed negative values of NEE that were even lower 21 (more carbon storage) that those found in control plots. These lower NEE values might be 22 explained by the rapid re-growth of the clipped vegetation, unhindered by light competition. 23 From this perspective, the reduction in GPP that we reported at our trampled plots can be 24 mostly explained by the impacts of trampling, which suppress regeneration and productivity 25 by destroying or damaging re-sprouting plants.

Natural peatlands tend to sequester  $CO_2$  and release  $CH_4$ , with a positive difference that turns these ecosystems into important carbon sinks(Haddaway et al., 2014; Veber et al., 2018; Xu et al., 2018). In our study, even low trampling intensity resulted in a switch from a carbon 1 sink to a carbon source, with greater impacts at higher trampling intensities. For our control

2 plots we found that the peatland has a  $CO_2$  uptake with a value of NEE of -0.77 g

3  $CO_2/m^2$ /hour. This values is similar to those found for undisturbed peatlands in Ecuadorian

4 Andes by Sánchez et al. (2017)(-0.69 g  $CO_2/m^2$ /hour), and by Planas-Clarke et al. (2020) for

5 inundated Peruvian Andes peatlands (-1.07 g  $CO_2/m^2$ /hour). As some evidence shows that

6 upland páramo soils could be turning into net carbon sources (Carrillo-Rojas et al. (2019),

7 probably as a result of climate change, our results highlight the importance of maintaining or

8 restoring páramo peatlands as a critical tool for climate change mitigation.

9 Other remarkable pattern of this study was the large increases in methane releases which, on

10 average, were between 3 and 10 times higher in the trampling plots, than in control plots. The

11 CH<sub>4</sub> emission that we report were considerable high, even in comparison to those found at an

12 Ecuadorian grazed peatland (Sánchez et al. (2017) (5.5 mg CH<sub>4</sub>/m<sup>2</sup>/hour), and at a Peruvian

13 Andean peatland (Planas-Clarke et al., (2020) (0.1 mg CH<sub>4</sub>/m<sup>2</sup>/hour). However, beyond the

14 increase in average CH<sub>4</sub> emissions, we also report a striking increase in the variation in these

15 measurements, which occurred only in the post-trampling phase of the experiment. This large

16 increase in the mean methane flux and the increase the variability suggest that methane

17 releases are not a constant and might occur in pulses or spatial patterns, probably related to

18 the heterogenous nature of the activity of the horses, which might release pockets of

19 accumulated methane in the subsoil (Limpens et al., 2008; Nazaries et al., 2013).

20 Another source of heterogeneity of the impacts of trampling on CO<sub>2</sub> and CH<sub>4</sub>, might be driven

21 by the type of vegetation and water-table levels. Although not shown here, information on

22 vegetation type on our sites, suggest that plots dominated by cushion or mat forming species

23 (*Plantago rigida, Lachemilla orbiculata*), have a much firmer structure, provided by the

24 density and compaction of the plant structure. In these plots, the hoofs of the horse used for

25 the experiment would not sink as easily, thus reducing the immediate effects of trampling. On

26 the contrary, at places with very high water-table levels and mostly dominated by *E*.

27 *dombeyana*, the hoofs easily broke the soil surface, disturbing the vegetation, and leaving

28 large patches of bare ground or mud. In this context, our study suggest that further studies

29 could evaluate the vulnerability peatland vegetation to the effects of cattle raising, by

30 assessing the impacts of trampling on different plant functional groups.

The influence of water table level is a phenomenon well-studied and in our analysis we found
 its influence mainly on the ecosystem respiration and methane fluxes which could be
 understood based on the reduction of the anoxic layer in the subsoil (Limpens et al., 2008).

Storing an average of more than 2000 Mg C/ha, páramo peatlands are a significant reservoir 4 5 of carbon (Benavides et al., 2023; Hribljan et al., 2017), and might play a disproportionate 6 role in water regulation (Mosquera et al., 2015). At the same time these ecosystems are highly 7 threatened by expanding agriculture, cattle raising, and unsustainable water extraction (Suarez 8 et al., 2022). On this broader scope, our study suggests two main conclusions: on one hand, 9 páramo peatlands are highly sensitive to trampling with significant changes in carbon storage 10 accruing even at low trampling intensities. These impacts seem to be driven by the destruction 11 of plant cover and the disruption of plant regeneration. On the other hand, as we carried out the experiment during the fifth year of a peatland restoration project (Suarez et al., 2022), our 12 13 data suggest that hydrological restoration has been highly effective in terms of promoting a 14 recovery of the carbon sequestration capacity of the peatland. In this context, our data suggest 15 that significant efforts are needed in terms of reducing the impacts of domestic and feral cattle 16 in páramo peatlands. Better management and additional peatland restoration initiatives seem 17 to be a highly promising direction in terms on protecting ecosystem services and mitigating 18 the effects of climate change in the northern páramos.

#### **5. CONCLUSION**

2 Trampling affects carbon dioxide fluxes in a huge way, normally transforming a carbon sink 3 peatland in a carbon source. However, the most important finding are the huge amounts of 4 Methane releases that are directly related with the physical disturbances and have a clear 5 impact on the climate change mitigation efforts. This change is mainly drive by the reduction 6 in the GPP of the ecosystems caused by the horse trampling. The presence of feral horses and 7 cows among the Ecuadorian peatlands even inside the national parks represent a huge threat 8 to conservation of the remanent peatlands. To better understand the impact of horse trampling 9 on physicochemical properties of the subsoil, it would be beneficial to conduct an experiment that either controls the variables or replicates the disturbance in a laboratory setting. 10

## **6. REFERENCES**

- Abdi, H. (2010). Holm's Sequential Bonferroni Procedure. *Encyclopedia of Research Design* Thousand Oaks, CA: Sage.
- Barros, A., & Pickering, C. M. (2015). Impacts of experimental trampling by hikers and pack animals on a high-altitude alpine sedge meadow in the Andes. *Plant Ecology & Diversity*, 8(2), 265–276. https://doi.org/10.1080/17550874.2014.893592
- Benavides, J. C., Vitt, D. H., & Cooper, D. J. (2023). The High-Elevation Peatlands of the Northern Andes, Colombia. *Plants*, 12(4), 955. https://doi.org/10.3390/plants12040955
- Blake G. R. (1965). Bulk density. University of Minessota.
- Carrillo-Rojas, G., Silva, B., Rollenbeck, R., Célleri, R., & Bendix, J. (2019). The breathing of the Andean highlands: Net ecosystem exchange and evapotranspiration over the páramo of southern Ecuador. *Agricultural and Forest Meteorology*, 265, 30–47. https://doi.org/10.1016/j.agrformet.2018.11.006
- Comas, X., Terry, N., Hribljan, J. A., Lilleskov, E. A., Suarez, E., Chimner, R. A., & Kolka, R. K. (2017). Estimating belowground carbon stocks in peatlands of the Ecuadorian páramo using ground-penetrating radar (GPR): Estimating Carbon Stocks in the Páramo. *Journal of Geophysical Research: Biogeosciences*, *122*(2), 370–386. https://doi.org/10.1002/2016JG003550
- Coronel, J. S., Declerck, S., Maldonado, M., Ollevier, F., & Brendonck, L. (2004). Temporary shallow pools in high-Andes 'bofedal' peatlands: *ARCHIVES DES SCIENCES*, 57; 85-96, http://doi.org/10.5169/seals-738439

Haddaway, N. R., Burden, A., Evans, C. D., Healey, J. R., Jones, D. L., Dalrymple, S. E., & Pullin, A. S. (2014). Evaluating effects of land management on greenhouse gas fluxes and carbon balances in boreo-temperate lowland peatland systems. *Environmental Evidence*, *3*(1), 5. https://doi.org/10.1186/2047-2382-3-5

Hribljan, J. A., Suarez, E., Bourgeau-Chavez, L., Endres, S., Lilleskov, E. A., Chimbolema,
S., Wayson, C., Serocki, E., & Chimner, R. A. (2017). Multidate, multisensor remote sensing reveals high density of carbon-rich mountain peatlands in the páramo of Ecuador. *Global Change Biology*, 23(12), 5412–5425.

https://doi.org/10.1111/gcb.13807

- Hribljan, J. A., Suárez, E., Heckman, K. A., Lilleskov, E. A., & Chimner, R. A. (2016).
  Peatland carbon stocks and accumulation rates in the Ecuadorian páramo. *Wetlands Ecology and Management*, 24(2), 113–127. https://doi.org/10.1007/s11273-016-9482-2
- Hutchinson, G. L., & Mosier, A. R. (1981). Improved Soil Cover Method for Field
  Measurement of Nitrous Oxide Fluxes. *Soil Science Society of America Journal*, 45(2),
  311–316. https://doi.org/10.2136/sssaj1981.03615995004500020017x
- Limpens, J., Berendse, F., Blodau, C., Canadell, J. G., Freeman, C., Holden, J., Roulet, N., Rydin, H., & Schaepman-Strub, G. (2008). Peatlands and the carbon cycle: From local processes to global implications – a synthesis. *Biogeosciences, 5, 1475-1491*. https://doi.org/10.5194/bg-5-1475-2008
- Mosquera, G. M., Lazo, P. X., Célleri, R., Wilcox, B. P., & Crespo, P. (2015). Runoff from tropical alpine grasslands increases with areal extent of wetlands. *CATENA*, 125, 120– 128. https://doi.org/10.1016/j.catena.2014.10.010

Nazaries, L., Murrell, J. C., Millard, P., Baggs, L., & Singh, B. K. (2013). Methane, microbes and models: Fundamental understanding of the soil methane cycle for future predictions: Methane, microbes and models. *Environmental Microbiology*, *15*(9), 2395–2417. https://doi.org/10.1111/1462-2920.12149

Picarro Inc. (2021). G4301 GasScouter Datasheet.

- Planas-Clarke, A. M., Chimner, R. A., Hribljan, J. A., Lilleskov, E. A., & Fuentealba, B. (2020). The effect of water table levels and short-term ditch restoration on mountain peatland carbon cycling in the Cordillera Blanca, Peru. *Wetlands Ecology and Management*, 28(1), 51–69. https://doi.org/10.1007/s11273-019-09694-z
- Sánchez, M. E., Chimner, R. A., Hribljan, J. A., Lilleskov, E. A., & Suárez, E. (2017). Carbon dioxide and methane fluxes in grazed and undisturbed mountain peatlands in the Ecuadorian Andes. *Mires and Peat*, 19, 1–18. https://doi.org/10.19189/MaP.2017.OMB.277
- Suarez, E., Chimbolema, S., Jaramillo, R., Zurita, L., Arellano, P., Chimner, R. A., Stanovick,
  J. S., & Lileskov, E. A. (2022). Challenges and opportunities for restoration of highelevation Andean peatlands in Ecuador. Mitig Adapt Strateg Glob Change 27(30), 17. https://doi.org/10.1007/s11027-022-10006-9
- Urbina, J. C., & Benavides, J. C. (2015). Simulated Small Scale Disturbances Increase Decomposition Rates and Facilitates Invasive Species Encroachment in a High Elevation Tropical Andean Peatland. *Biotropica*, 47(2), 143–151. https://doi.org/10.1111/btp.12191
- Veber, G., Kull, A., Villa, J. A., Maddison, M., Paal, J., Oja, T., Iturraspe, R., Pärn, J., Teemusk, A., & Mander, Ü. (2018). Greenhouse gas emissions in natural and

managed peatlands of America: Case studies along a latitudinal gradient. *Ecological Engineering*, *114*, 34–45. https://doi.org/10.1016/j.ecoleng.2017.06.068

- Whymper, E. (1891). TRAVELS AMONGST THE GREAT ANDES OF THE EQUATOR. CHARLES SCRIBNER'S SONS.
- Xu, J., Morris, P. J., Liu, J., & Holden, J. (2018). PEATMAP: Refining estimates of global peatland distribution based on a meta-analysis. *CATENA*, 160, 134–140. https://doi.org/10.1016/j.catena.2017.09.010
- Yu, Z. (2011). Holocene carbon flux histories of the world's peatlands: Global carbon-cycle implications. *The Holocene*, 21(5), 761–774. https://doi.org/10.1177/0959683610386982
- Yuan, F., Wang, Y., Ricciuto, D. M., Shi, X., Yuan, F., Brehme, T., Bridgham, S., Keller, J.,
  Warren, J. M., Griffiths, N. A., Sebestyen, S. D., Hanson, P. J., Thornton, P. E., & Xu,
  X. (2021). Hydrological feedbacks on peatland CH4 emission under warming and
  elevated CO2: A modeling study. *Journal of Hydrology*, 603, 127137.
  https://doi.org/10.1016/j.jhydrol.2021.127137

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## 8. TABLES

## Table 1.

The Coefficients (Coef), Standard Errors (SE), and P Values (P > z) for All Significant Terms in the GLM Models

	Net Ecos $R^2 = 0.42$	system Ex 262	change	Ecosystem Respiration $R^2 =$ 0.3379		Methane $R^2 = 0.2933$			
Variables	Coef	SE	р	Coef	SE	р	Coef	SE	р
High	0.0218	0.0022	***	-0.0008	0.0004		0.0019	0.0005	***
Medium	0.0182	0.0022	***	-0.0006	0.0005		0.0013	0.0005	*
Low	0.0162	0.0022	***	-0.0002	0.0005		0.0017	0.0005	**
WTL	-0.0022	0.0012		0.0026	0.0002	***	-0.0015	0.0003	***
PAR	-0.0048	0.0009	**	0.0004	0.0002		0.0003	0.0002	
High:WTL	0.0028	0.0033		0.0008	0.0007		-0.0012	0.0007	
Medium:WTL	-0.0034	0.0033		0.0009	0.0007		-0.0010	0.0007	
Low:WTL	0.0000	0.0032		0.0012	0.0007		-0.0012	0.0007	
High:PAR	0.0072	0.0024	*	0.0004	0.0006		0.0002	0.0006	
Medium:PAR	0.0030	0.0024		0.0004	0.0006		-0.0007	0.0006	
Low:PAR	0.0016	0.0023		0.0002	0.0006		0.0008	0.0006	
Hig:PAR:WTL	-0.0014	0.0040		0.0002	0.0009		-0.0010	0.0010	
Medium:PAR:WTL	-0.0060	0.0037		0.0008	0.0009		0.0004	0.0009	
Low:PAR:WTL	-0.0072	0.0040		0.0004	0.0009		0.0003	0.0010	
	Net Ecosystem Exchange $R^2 = 0.3926$		Ecosystem Respiration $R^2 = 0.0270$		Methane $R^2 = 0.0270$				
	Coef	SE	р	Coef	SE	р	Coef	SE	р
Trampling	0.0137	0.0016	***	0.0007	0.0003	*	0.0006	0.0003	***
High	0.0161	0.0019	***	-0.0008	0.0004		0.0013	0.0004	***
Medium	0.0132	0.0019	***	-0.0003	0.0004		0.0010	0.0004	**
Low	0.0120	0.0019	***	-0.0001	0.0004		0.0013	0.0004	***

High:Trampling	0.0223	0.0038	***	-0.0001	0.0009	0.0019	0.0008
Medium:Trampling	0.0193	0.0038	***	-0.0001	0.0009	0.0012	0.0008
Low:Trampling	0.0158	0.0037	***	-0.0001	0.0009	0.0013	0.0008

*Note:* High = High intensity trampling; Medium = Medium intensity trampling; Low = Low intensity trampling; WTL = Water table level; PAR = Photosynthetically active radiation; \*\*\* denotes p < 0.001; \*\* denotes p < 0.01; \*\* denotes p < 0.01; \*\* denotes p < 0.05.

#### Table 2.

Pairwise comparisons using t tests for the NEE measures with pooled Standard Desviation

Treatment Intensity	Control	High	Medium
High	0.000	-	-
Medium	0.000	0.202	-
Low	0.000	0.037	0.390

Note: The Holm method was used to calculate the familywise error rate

### Table 3.

Pairwise comparisons using t tests for the CH<sub>4</sub> fluxes with pooled Standard Desviation

Treatment Intensity	Control	High	Medium
High	0.000	-	-
Medium	0.000	1.000	-
Low	0.000	1.000	1.000

Note: The Holm method was used to calculate the familywise error rate

#### Table 4.

The p-values and Cohen d factors for paired T-tests of bulk density and soil carbon content comparing the pre-trampling with posttrampling

Treatment	Section	Bulk	Density	Soil Carbon Content		
Intensity	Section -	р	Cohen d	р	Cohen d	
High	0-5		0.011		0.298	
	5-10	***	0.672		0.202	
Medium	0-5		0.637		0.064	
	5-10		0.301		0.142	
Low	0-5		0.298		0.053	
	5-10		0.477		0.103	
Control	0-5		0.546		0.167	
	5-10	***	0.875		0.020	

## 9. FIGURES





Figure 1 Chakana peatland location in the northern andes of Ecuador and distribution of water table blocks across the peatland.

Figure 2.



Figure 2 Schematic representation of the distribution of the four intensity plots inside each block.

### Figure 3.



Figure 3 Francisco Cuichán, the reserve ranger executing the horse trampling.



## Figure 4.

Figure 3 Mean Gross Primary Production (GPP), Ecosystem Respiration, and Net Ecosystem Exchange for the pre- trampling phase (A-B-C-D) and for post-trampling phase (E-F-G-H) distributed by trampling intensity. Error bars indicate one standard error. Values are represented in g  $CO_2 m^{-2} hour^{-1}$ and negative values represent carbon sequestration by the ecosystem, positive values represent carbon release from the ecosystem.

## Figure 5.



Figure 4 Mean Gross Primary Production (GPP), Ecosystem Respiration, and Net Ecosystem Exchange for the extra control plot. Error bars indicate one standard error. Values are represented in g  $CO_2 m^{-2}$  hour<sup>-1</sup> and negative values represent carbon sequestration by the ecosystem, positive values represent carbon release from the ecosystem.



#### Figure 6.

Figure 5 Mean Methane fluxes by intensity and with pre-trampling (red) and post-trampling (blue) comparison distributed by trampling intensity. Error bars indicate one standard error. Values are represented in mg  $CO_2 m^{-2} hour^{-1}$ .





Figure 6 Methane fluxes across the time during the experiment from April 2022 until March 2023 in a logarithmic scale for the pre-trampling phase (red dotes) and post-trampling phase (blue dots) distributed by trampling intensity. Values are represented in mg  $CO_2 m^{-2} hour^{-1}$ .

#### Figure 8.



Figure 8 Bulk density for pre-trampling phase (green dots) and post-trampling phase (yellow dots) distributed by trampling intensity for the first section of soil sample (0-5 cm). Values are represented in  $g \text{ cm}^{-3}$ .





Figure 9 Bulk density for pre-trampling phase (green dots) and post-trampling phase (yellow dots) distributed by trampling intensity for the first section of soil sample (0-5 cm). Values are represented in g cm<sup>-3</sup>.