

A high CO₂ pressure-based method for soil microbial cells disruption

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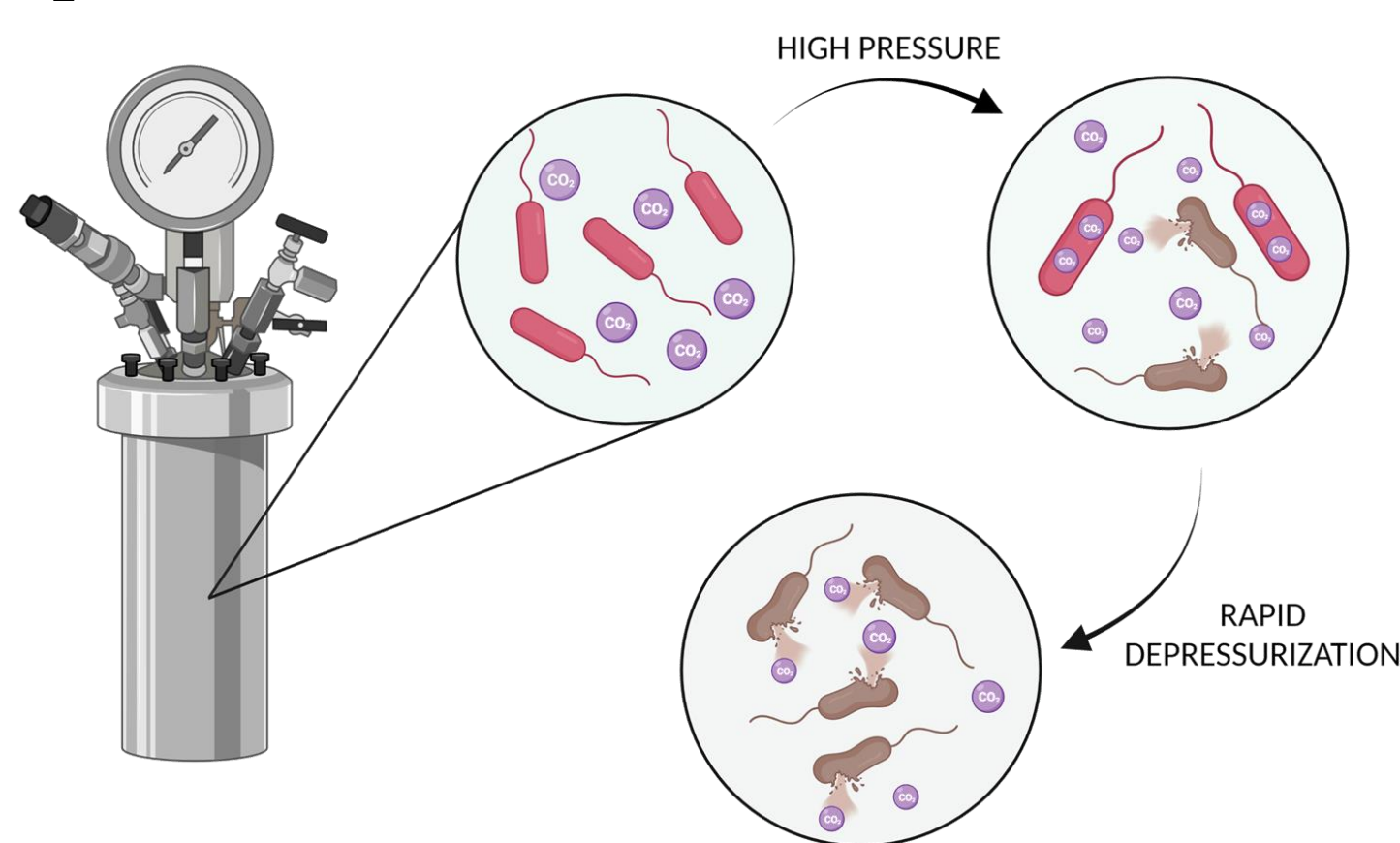
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INTRODUCTION

Soil microbial biomass (SMB), a small but highly dynamic pool of living organic matter, plays a key role in nutrient cycling, and therefore its size and activity are crucial determinants of soil fertility and quality [1]. For these reasons, the study and characterization of soil microbial biomass (SMB) are essential for soil quality assessment. The two methods still widely used for SMB determination are chloroform fumigation incubation (FI) and chloroform fumigation extraction (FE) [2][3]. Both methods rely on the ability of chloroform (CHCl₃) to lyse soil microbial cells so as to determine the released cytoplasmic material. The use of CHCl₃, however, raises several critical issues, chief among them that it is toxic to humans and the environment [4]. In addition, several authors have shown that CHCl₃ is not completely efficient in lysing microbial cells [5][6][7].

AIM OF THE STUDY

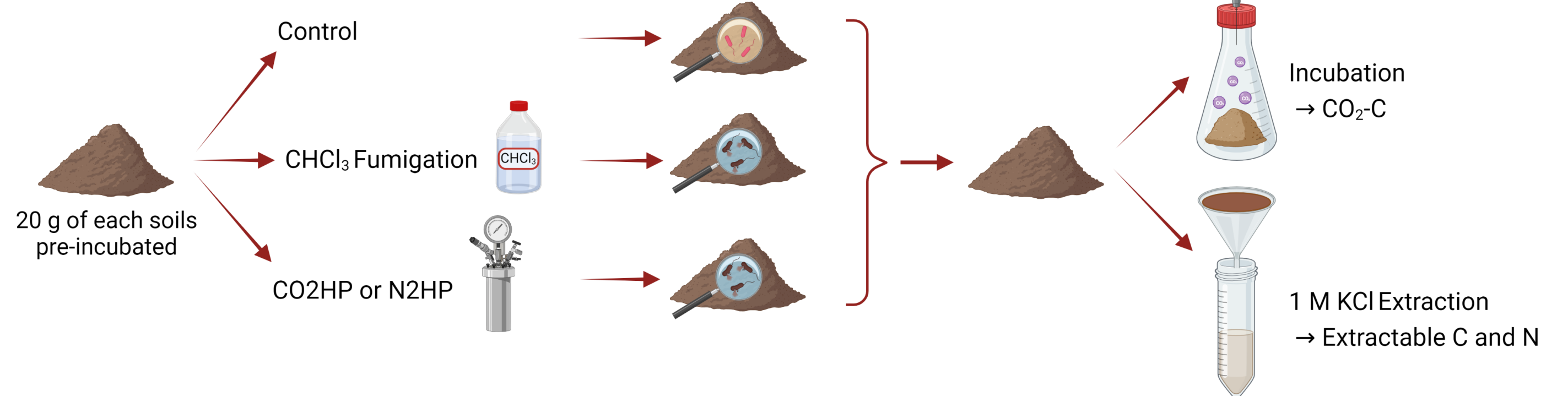
The aim of this study was to develop a new approach, possibly more reliable and environmentally safe than the CHCl₃-based method, for lysing soil microbial cells. The proposed method is based on high pressurization of the soil with CO₂, through the use of a steel reactor, followed by rapid depressurization through gas release. Hereafter, we will call this approach CO₂HP (CO₂-High Pressure).



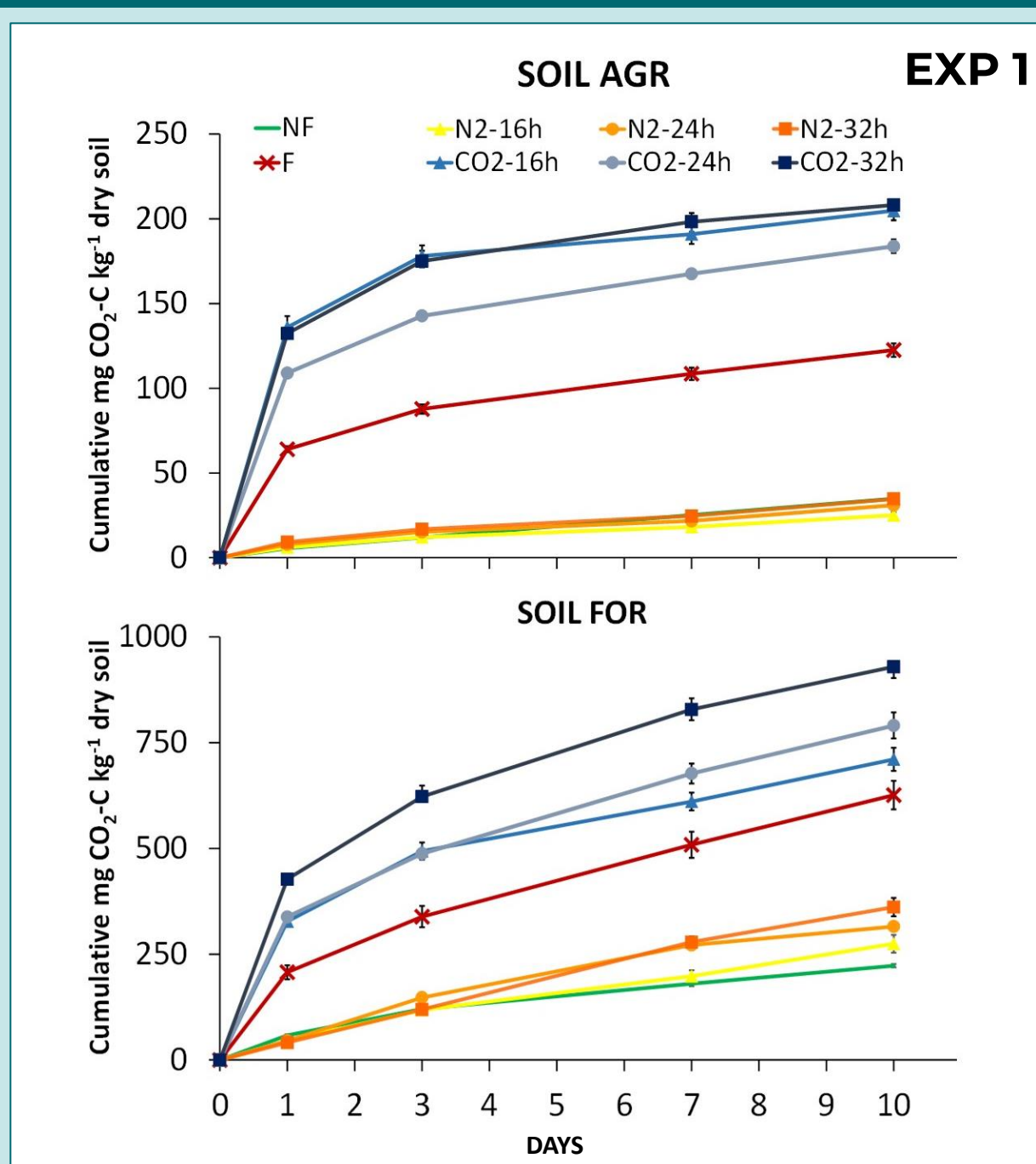
METHODS

EXP	Comparison	Constant parameter	Variable parameter
1	N2HP vs. CFI	600 psi	16, 24, 32 hours
	CO2HP vs. CFI	600 psi	16, 24, 32 hours
2	CO2HP vs. CFE	4 hours	400, 500, 600, 700, 800 psi
		24 hours	400, 500, 600, 700, 800 psi
3	CO2HP vs. CFE	500 psi	2, 4, 6, 8 hours
		600 psi	16, 24, 32 hours

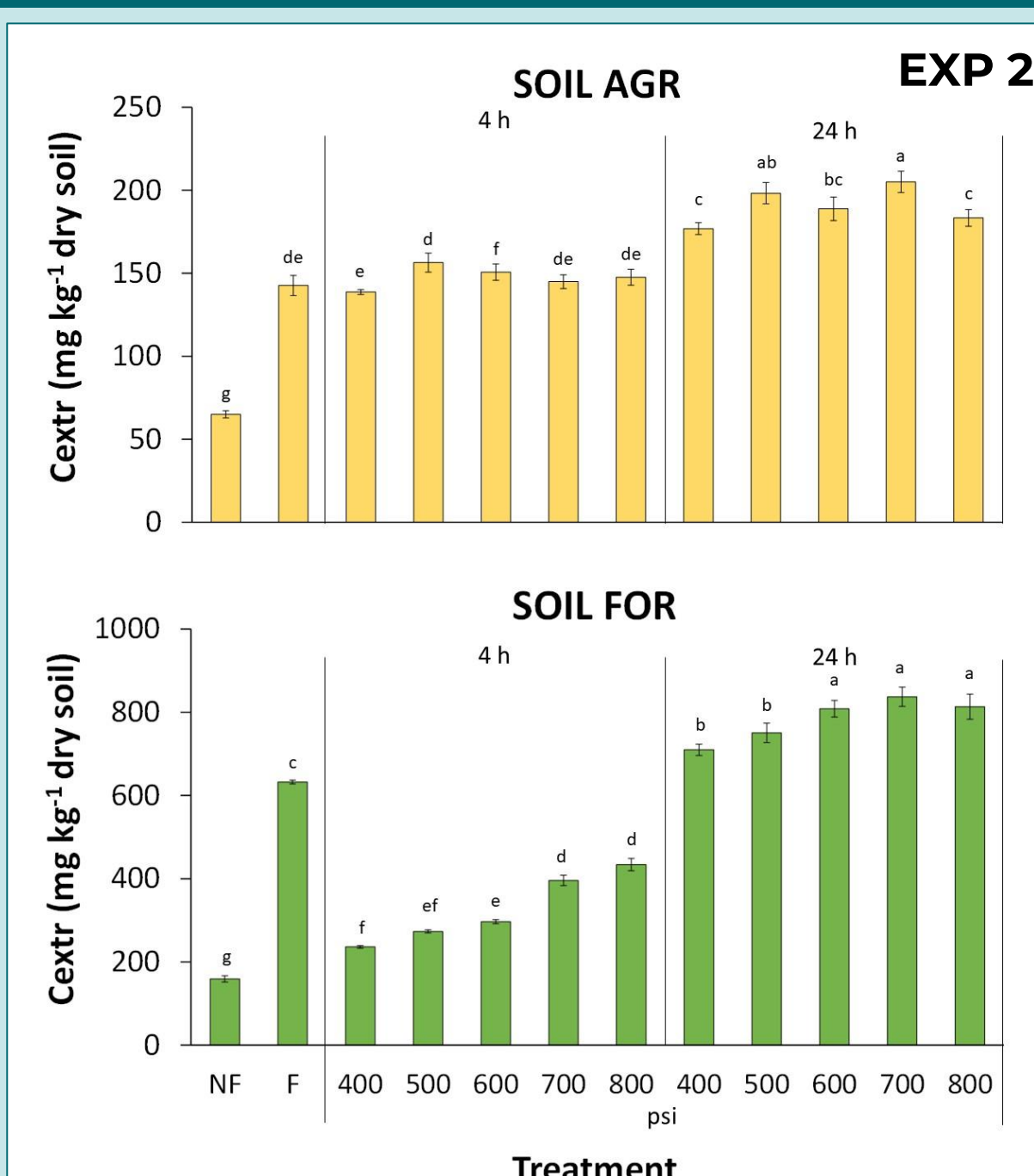
To set up CO₂HP method, different combinations of pressure and duration of pressurization were tested, and a comparison was made with the classical FI and FE methods to evaluate the ability of the CO₂HP method to lyse soil microbial cells.



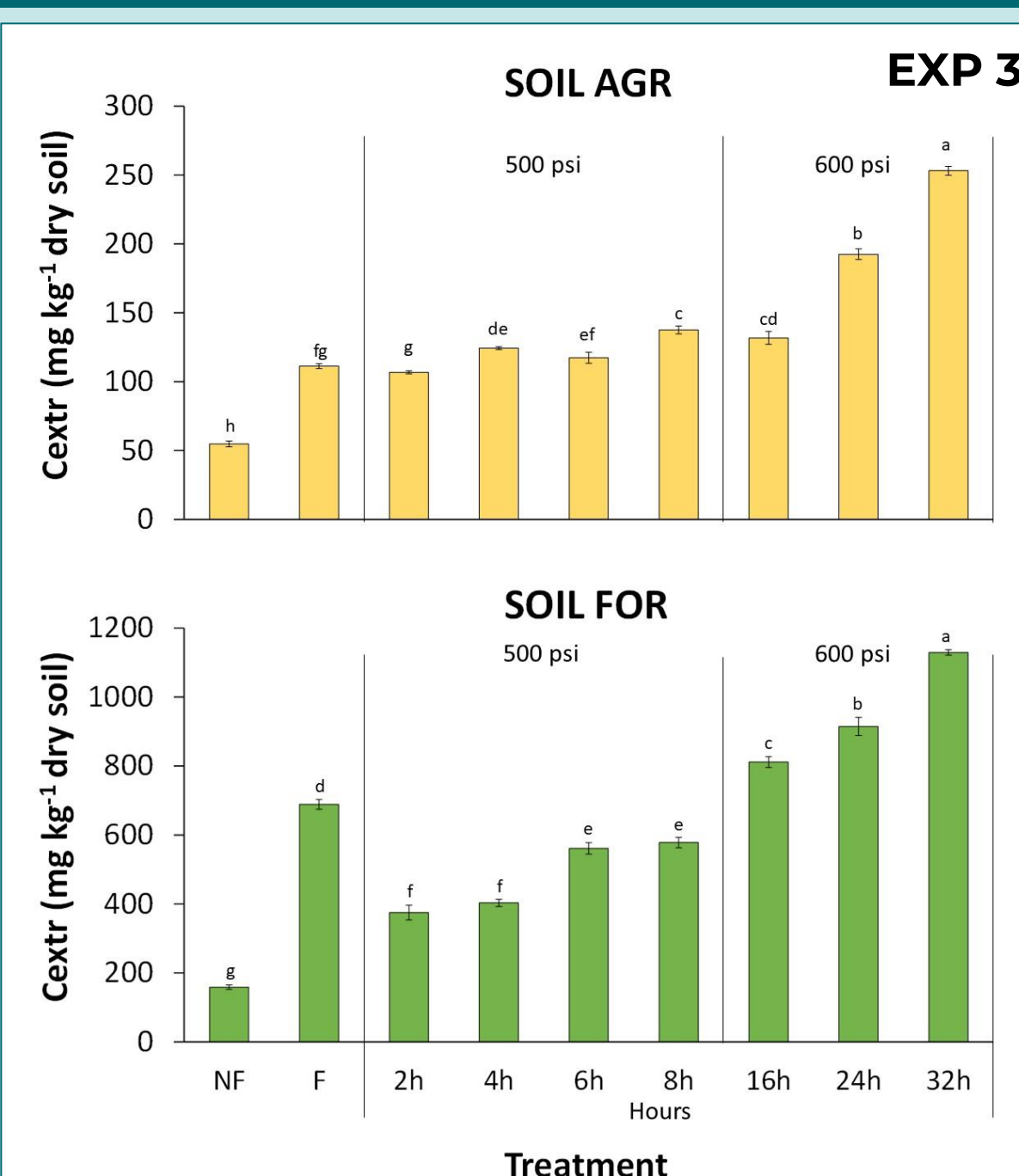
RESULTS



EXP 1. Cumulative CO₂ emitted from AGR and FOR soils during a 10-days incubation and subjected to constant high pressure (600 psi) for 16, 24, and 32 h with N₂ (N2HP) or CO₂ (CO2HP). NF, not fumigated (control); F, CHCl₃ fumigated. Values represent means ± SD (n = 4).



EXP 2, 3. KCl-extractable C from AGR and FOR soils subjected to various high CO₂ pressures (from 400 to 800 psi) and various durations (from 2 to 32 hours) (CO2HP). NF, not fumigated (control); F, CHCl₃ fumigated. Values represent means ± SD (n = 4). Different lowercase letters indicate significant differences at P<0.05



The cumulative amount of CO₂-C (C_{flux}) evolved during 10 days of incubation from both soils pressurized with N₂ was much lower than that of fumigated soil, suggesting that N₂ is not suitable for lysing cells (EXP 1). C_{flux} produced by soil pressurized with CO₂ (600 psi) was much higher than that of CHCl₃ fumigated soils. C_{flux} produced by FOR soil pressurized with CO₂ (600 psi) increase by increasing pressurization time (EXP 1). The results of EXP 2 and 3 suggest that more microbial cells could be lysed with the CO₂HP method at pressures greater than 500 psi and durations longer than 16 h than with CHCl₃-fumigation.

CONCLUSIONS

- The **CO₂HP** technique was **more effective than CHCl₃ fumigation** in lysing soil microbial cells.
- As a general rule, the higher the soil **organic carbon content**, and likely microbial biomass, the **higher both CO₂ pressure and the pressurization time** to disrupt microbial cells.
- The most successful combination of high CO₂ pressure and duration was **600 psi x 32 hours**.
- Future work:** to both validate and standardize the CO₂HP approach as a new method for determining SMB, further studies are essential using soils added with known amounts of ¹³C- and/or ¹⁵N-labeled microbial biomass.

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