

Exploring factors influencing volatile compounds in fecal marking of free-ranging Iberian wolves: diversity, substrates and time

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INTRODUCTION & OBJECTIVES

The Iberian wolf (*Canis lupus signatus*) is an apex predator mostly in the north of the Duero River (Fig 1). Wolves are heavily territorial species that live in packs formed by a reproductive pair. These individuals are the only ones who show marking behavior [1]. Studies show that wolves often mark the same trail intersections [2, 3] and that the function of feces as chemical signals lies in the great number and diversity of volatile compounds they contain [1, 3]. Although the identification of volatile compounds in scent marks is now possible, it remains unclear to what extent the physical properties of the substrates selected for marking influence the persistence of these chemical signals.



Figure 1. *C. lupus signatus* pup photographed during fecal sample collection in the park (Photo by Isabel Barja).

AIM → Understand the factors influencing the duration and effectiveness of feces as chemical signals in the Iberian wolf (*Canis lupus signatus*). We address the following questions:

1. Do volatile compounds are different depending on the **exposure time** since deposition?
2. Do volatile compounds vary depending on the **type of substrate** on which they were deposited?
3. Do **temperature** differences in each substrate affect the volatile compounds in the feces?

RESULTS & DISCUSSION

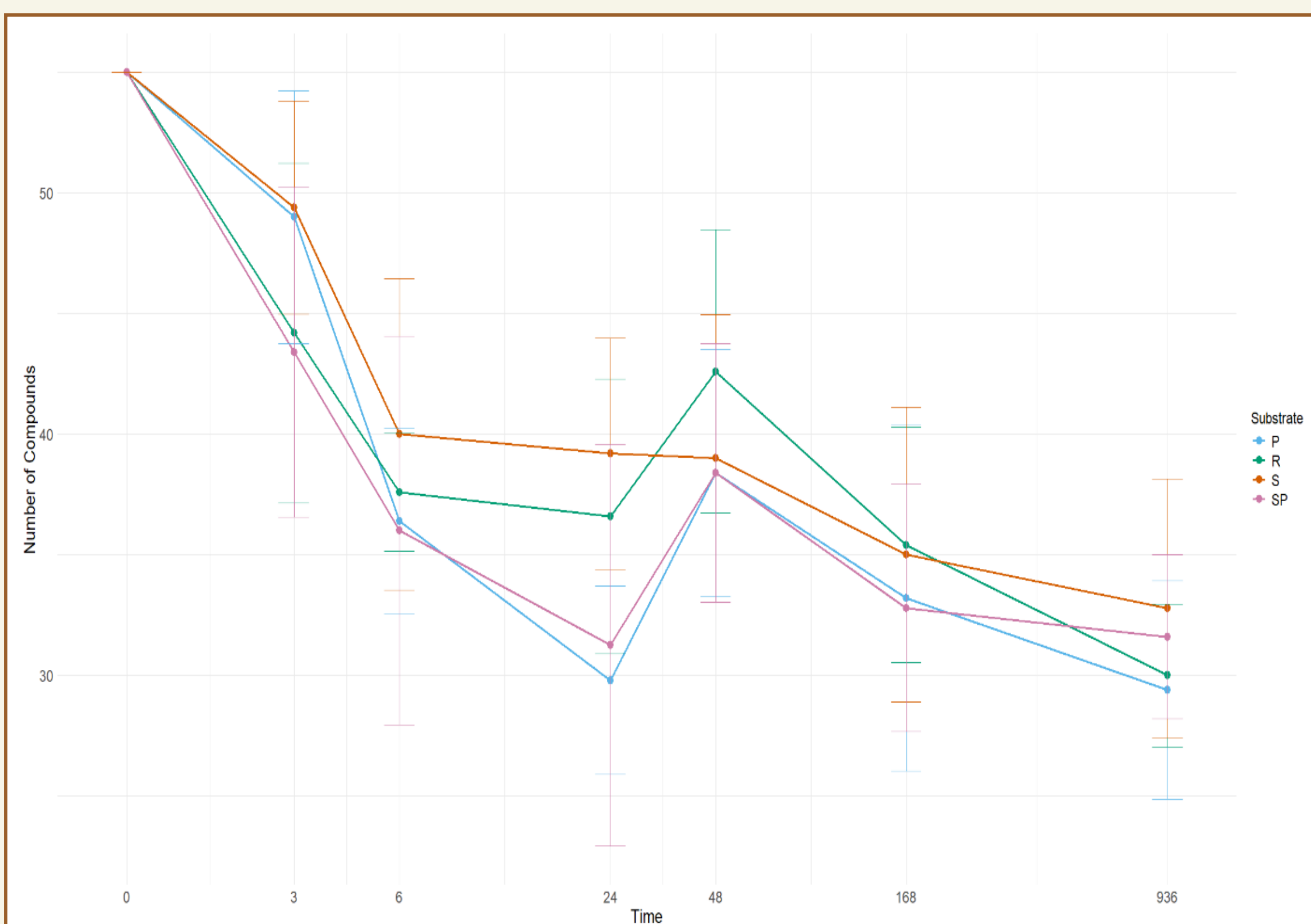


Figure 2. Mean number of compounds per sample based on the time-period. Points indicate the mean number of compounds based on different time-period in hours. Each line type indicates a different substrate (P = Plant, R = Rocks, S = Sand and SP = Sand Prominence).

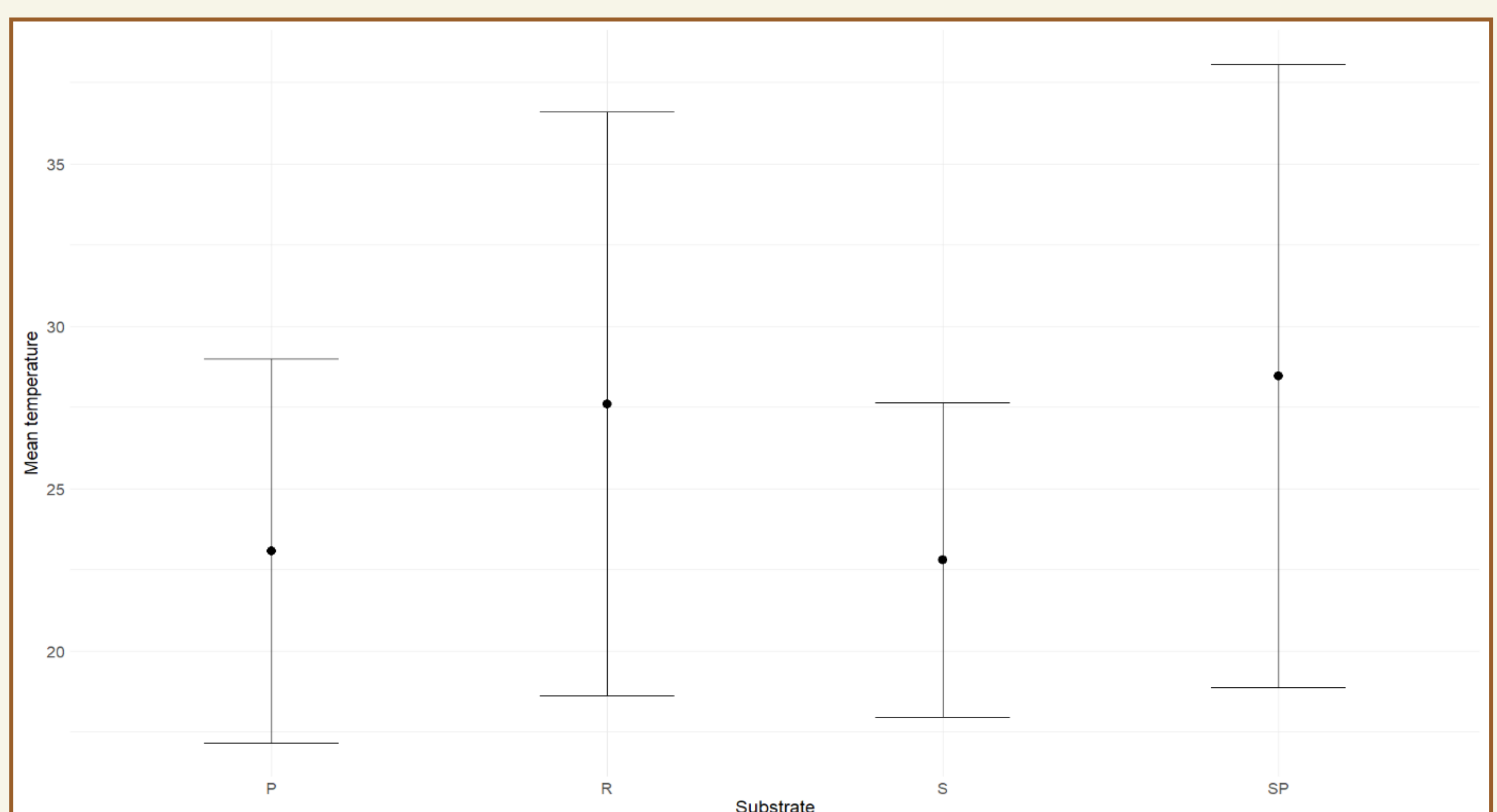


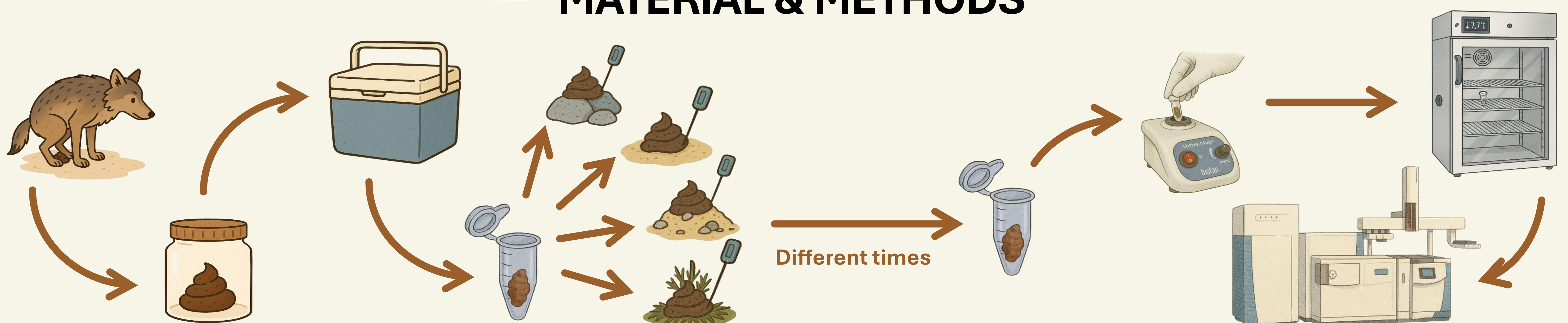
Figure 3. Mean temperature of substrates measured in the experiment. Points represent the mean temperature for different substrates (P = Plant, R = Rocks, S = Sand, and SP = Sand Prominence).

CONCLUSIONS

- Volatile compounds vary depending on different environmental conditions and may play an important role in fecal marking.
- Volatile-rich marks on sand prominent substrates facilitate rapid intra-group communication, whereas more stable marks on plants or sand support long-lasting, energy-efficient territorial signaling.
- Cooler substrates like plants allow gradual volatile release, and ground-level marks, especially at boundaries or intersections, may maximize signal persistence or visibility depending on the substrate type.

- The number of compounds was significantly higher at early time points and decreased at later time points (Fig. 2), since scent marks in the Iberian Peninsula are subjected to air and intense sunlight, which presumably degrades rapidly chemical compounds.
- An elevated value was observed at 48 h probably due to a slight increase in new compounds produced by bacteria involved feces' degradation.
- The number of compounds was significantly different for rock, sand prominence and plant, probably because of the different temperatures the substrates reach.
- Temperature varied significantly among substrates, with sand prominence reaching the highest temperatures and plant and sand the lowest (Fig 3). While rocks of the study area retain heat, plants have evolved to adapt and tolerate heat stress.

MATERIAL & METHODS



References: [1] Barja, I., Piñeiro, A., Ruiz-González, A., Caro, A., López, P., & Martín, J. (2023). Evaluating the functional, sexual and seasonal variation in the chemical constituents from feces of adult Iberian wolves (*Canis lupus signatus*). *Scientific Reports*, 13(1). [2] Vilà, C., Urios, V., & Castroviejo, J. (1994). Use of faeces for scent marking in Iberian wolves (*Canis lupus*). *Canadian Journal of Zoology*, 72(2), 374-377. [3] Martín, J., Barja, I., & López, P. (2010). Chemical scent constituents in feces of wild Iberian wolves (*Canis lupus signatus*). *Biochemical Systematics and Ecology*, 38(6), 1096-1102.

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