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# Finding the optimal strategy for quantitative sampling of springtails community (Hexapoda: Collembola) in glacial lithosols

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ABSTRACT

Springtails (Hexapoda: Collembola) play a key role in biological community in glacial ecosystems and represent important ecological indicators in such threatened habitats. An effective sampling protocol for springtail community that optimizes sampling effort in the peculiar conditions of glacial lithosols is not available. We tested three sampling methods on the Sforzellina glacier (Central Italian Alps) in 21 sampling points. For each sampling point, we performed: 1. Tullgren funnels; 2. Flotation method; 3. Pitfall trapping. The potential effect of different sampling methods on species detection was evaluated by ANOVA and by N-mixture models for Flotation method and Tullgren funnels. The incidence coverage estimator (ICE) was used to test the performance of each sampling method comparing the observed vs estimated species richness. Our analysis showed that the sampling method affected the number of species and individuals recorded. Tullgren funnels collected the highest number of species, pitfall trapping the highest average number of species, but did not detect soil species. The observed/estimated species ratio was higher for pitfall trapping and Tullgren funnels than for flotation. The combination of pitfall trapping with Tullgren funnels or flotation method resulted optimal in terms of number of species and functional types recorded. Flotation method collected more than twice the number of specimens obtained with Tullgren, indicating a higher ability to extract springtails from mineral soil. Flotation method and Tullgren funnels detected the same community, from a functional point of view, but only flotation method collected all the most abundant species. These results indicate that a combination of pitfall trapping and flotation should be evaluated in order to maximize the obtained information in terms of specie assemblage composition and functional categories.

## 1. Introduction

Springtails (Hexapoda: Collembola) are a widespread group of arthropods, able to colonize almost all terrestrial habitats; they are particularly abundant in soil (Hopkin, 1997). They have a central importance in the biological community and food web in species-poor and stressed glacial habitats, from Alpine (Buda et al., 2020; Hågvar et al., 2020; Hågvar and Gobbi, 2022; Valle et al., 2022a) to Arctic and Antarctic glacial sites (Krab et al., 2013; Beet et al., 2022; Hågvar, 2010), where they act as pioneer organisms. This pioneer profile is linked to their wide range of thermal tolerance (Block and Zettel, 1980; Beet et al., 2022), their positive link to cold biomes (Potapov et al., 2022) and to an opportunistic feeding habits (Beet et al., 2022).

Alpine glaciers are currently shrinking and threatened by climate change and a deep knowledge and a monitoring of the biodiversity and ecology of this habitat is needed (Gobbi et al., 2021). Due to their adaptation to cold environments, alpine springtails are potentially good sentinels of climate change, specifically cryophilic (i.e. adapted to cold and humid micro-habitat) species in Alpine glacial habitat (Valle et al., 2021, 2022a,b). So far, few works studied springtails in glacial habitat (e.g., Haybach, 1972, Buda et al., 2020; Hågvar, 2010, Hågvar et al., 2020, Valle et al., 2021, 2022a; b). A large-scale knowledge of the

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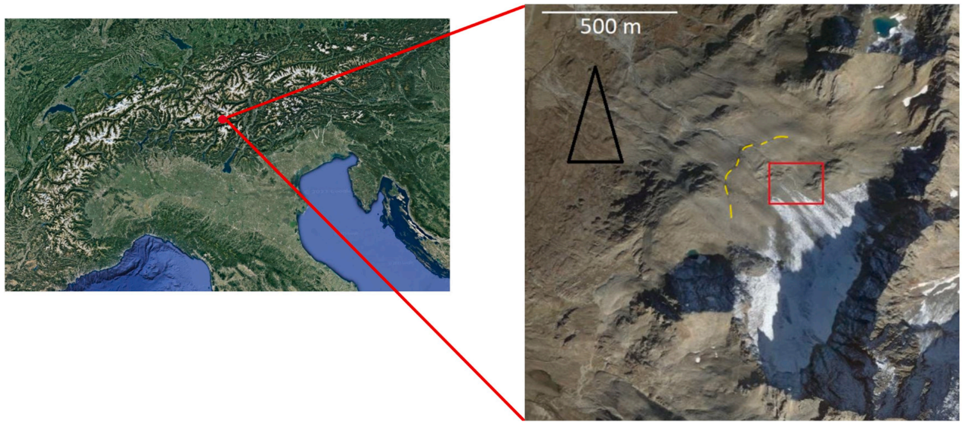


**Fig. 1.** Study area: Sforzellina glacier in Stelvio National Park (SO, Italy). Yellow dashed line: glacier moraine of the 1989 advance. Red rectangle shows the area detailed in Fig. 2A. (
Source: Google Earth).

species assemblages in as much as possible Alpine glaciers is mandatory because these glaciers are shrinking and sometimes vanishing and, with them, their glacial springtails' biodiversity. On the other hand, to reach this ambitious goal, a standard and cost-efficient sampling method for describing and monitoring glacial springtail communities, particularly those of lithosols on recently deglaciated terrains and in the stony debris above the ice, is still missing.

Springtails can be categorized in a broad spectrum of functional categories, from atmobiotic and epiedaphic (i.e. "ground-dwelling springtails"), hemiedaphic and euedaphic species (i.e., "soil springtails") (Potapov et al., 2016). Consequently, the sampling protocol should include sampling methods able to investigate all these components.

Previous studies demonstrated that the combination of extraction of soil samples and pitfall trapping is the most efficient sampling strategy for detecting diversity and species composition of springtails in agricultural habitats (Querner and Bruckner, 2010; Nsengimana et al., 2017). One of the most effective method for extracting soil fauna is the Berlese-Tullgren funnels (Marshall et al., 1994; hereafter "Tullgren funnels"), which consists in separating arthropods from a soil sample using heat and desiccation that induce migration of organism toward a

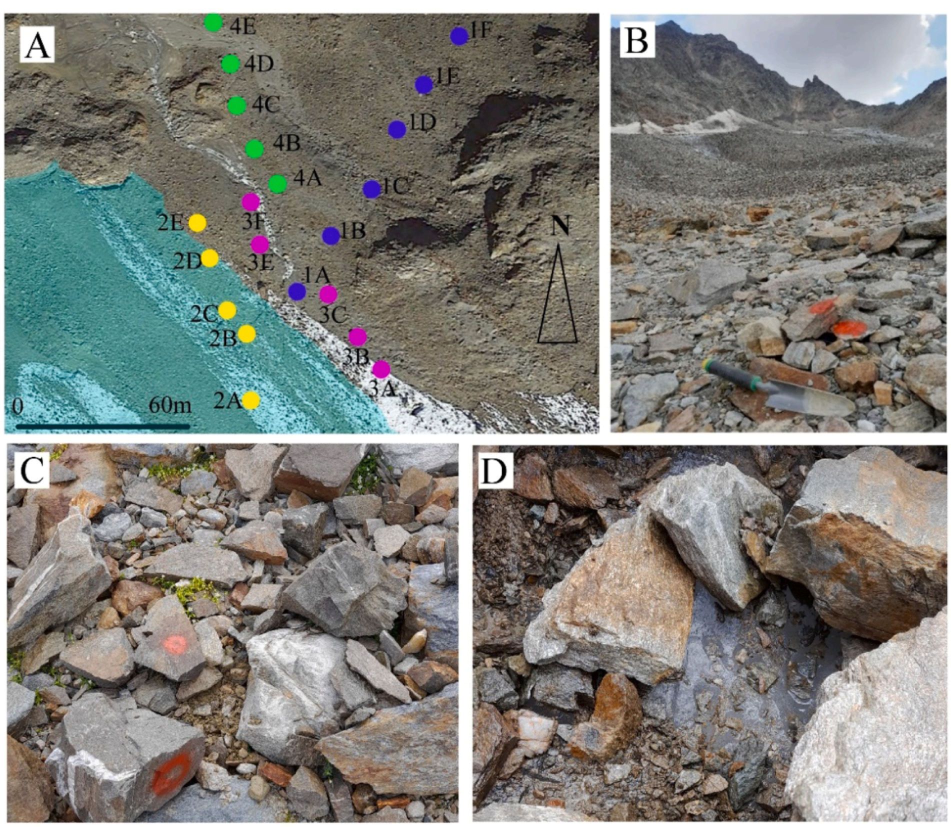


**Fig. 2.** (A) Sampling plan on the Sforzellina glacial habitats. Light-blue polygon= ice tongue in 2022, corresponding to the supraglacial habitat (orthophoto from September 2021. (B) Sforzellina glacier viewed from SFO3E; (C) example of sampling point from a site along the glacier foreland (SFO1D); (D) example of sampling point on supraglacial habitat, with surfacing ice (SFO2D)
Source: Google Earth).

trapping device. This method is usually very effective for springtail communities in stable and mature soils (e.g., Kuznetsova, 2003), but mainly active, free-living stages are extracted (Marshall et al., 1994; Jureková et al., 2021). Tullgren funnels has been successfully used along Arctic glacier forelands (Hågvar, 2010), but its use in lithosols is poorly experienced in literature (e.g., Raschmanová et al., 2013). For mineral and dry soils, Kethley (1991) proposed another technique for extracting soil fauna, the flotation method, which is carried out washing a soil sample in water (Kethley, 1991) or in other solutions (e.g., in heptane, Walter et al., 1987), actively separating the organisms with hydrophobic cuticle from the soil samples (Marshall et al., 1994). Both these methods – flotation method and Tullgren funnels - can provide quantitative data as they are applied on known soil volumes. They are used for extracting mainly soil springtails, but are less efficient with ground-dwelling springtails (e.g., Querner and Bruckner, 2010). An effective method for collecting ground-dwelling springtails are the pitfall traps, widely used also along glacier foreland (Hågvar et al., 2020; Gobbi and Lencioni, 2020); also pitfall traps can provide quantitative data being active for a known time interval, but unlike the previous methods, such data cannot be quantified for a known surface/volume of soil.

Working in glacial environments located at high elevation needs to draw a sampling protocol as much as possible easy with regard to time-effectiveness, labor intensity, and storage of specimens easy (i.e. no heavy or bulky equipment), because of the overall physical effort to reach glacial habitats, weather instability, increasing amount of disturbance events such as debris flows and flooding, which limit the available time for an exhaustive sampling. Limiting the number of sampling sessions and the time span spent on the glacier, while obtaining a valuable amount of information, is of paramount importance when investigating such habitats.

On the base of the above reported needs and threats, the aim of our work was to test the performance of three standard methods (Tullgren funnels, flotation method and pitfall trapping) in order to find the most effective method (or combination of methods) for sampling and analyzing springtail communities (i.e. species richness, community composition) in glacial environments, on inconsistent and often unstable lithosols. To reach this goal we investigated the springtails community of a vanishing glacier of the Central Italian Alps.

## 2. Material and methods

### 2.1. Study area

Sampling work was performed on supraglacial and proglacial areas deglaciated less than 30 years ago of the Sforzellina glacier, at about 2800 m a.s.l. Sforzellina glacier (46°20'56.9"N 10°30'44.2"E) is located on Ortles-Cevedale Massif within the Stelvio National Park (Southern Rhaetian Alps, European Alps, Italy) (Fig. 1).

The glacier covers a surface of 0.22 km$^2$ (Paul et al., 2020; data of 2016) and the glacial front is located at 2835 m asl (field data recorded in 2022). Sforzellina glacier has a North-West aspect and is surrounded by the Corno dei Tre Signori peak (3376 m s.l.m.) and other lower peaks constituted by phyllites, micaschist and paragneiss (Chiesa et al., 2011).

Stony debris currently covers more than the 50 % of the ice surface and is mainly composed by angular clasts of metamorphic rocks coarser than 25 mm (Tarca and Guglielmin, 2022). The area is characterized by continental Alpine climate (Soncini et al., 2017).

### 2.2. Sampling design

Four linear transects have been positioned in order to cover the micro-habitat variability among the investigated plots (Fig. 1, 2). In particular, supraglacial environment and proglacial areas deglaciated less than 30 years ago (included within the moraine of the advance of the 1989) were sampled. The transect is not a sampling unit, but a practical solution in order to make easier the finding of the sampling sites in rocky substrates. All the sampling points are random replicates placed in both supraglacial and proglacial environments.

Supraglacial debris had a maximum depth of 20 cm. Elevation is comparable among all the sampling sites.

Along every transect, five (six in transect 1) sampling points were selected, spaced about 15 m from each other. Totally, 21 sampling points were placed. On the supraglacial habitat four sampling points were placed (SFO $^2$A–$^2$B-2 C-2D), while all other sampling points were placed in the proglacial habitat. In each sampling point we used: (i) Tullgren funnels (Dritsoulas and Duncan, 2020): samples for Tullgren funnels were taken on 20 August 2022. In particular, for each sample, a plastic glass (diameter 7 cm, height 8 cm, 200 ml) was filled with soil, trying to maintain the soil structure of the inconsistent debris; then the glass was closed with tape and the samples preserved in cool chamber (at 4 °C) until the extraction (within 7 days from the sampling). The equipment is practical and transportable (and easy to use in a mountain hut) and consists of a plastic funnels with a net (grid of about 1.5–2 mm) fixed at the opening, where the sample is placed (Supplementary Fig. S1). The structure supporting the funnels is a cylinder obtained by a plastic bottle, cut at the edge, easy to build and to use. Under the funnels, a tube filled with alcohol 96° is placed. The device is placed about 30 cm under a light and heat source (incandescent light bulb). Samples were removed from Tullgren funnels when completely dry, after at least 10 h: the short time needed depends on the characteristics of the mineral soil (see Supplementary Figs. S2A-B), which after few hour is already completely dry.; (ii) Flotation method (Marshall et al., 1994, Valle et al., 2022a,b): it was performed on 20 July 2022 on debris samples collected near the pitfall traps with a scoop and placed in a plastic glass of the same dimensions used for Tullgren funnels method. This quantity of debris was screened for arthropods in a small basin filled with mineral water (ratio of water to soil: about 4:1), moving the debris with the scoop, in order to move organisms hidden in the debris and make them float. The springtails were sampled with a small brush or a stick (lifting them carefully from the water surface) and put directly in a tube with alcohol 96°. The sample should be screened until every individual is collected (the duration of the flotation method is variable in relation to the number of individuals present, but it is important to screen the sample and move the debris for at least 1 min); iii) Pitfall trapping: a pitfall trap was set up, consisting of a plastic glass of the same dimensions used for flotation method and Tullgren funnels, levelled with the ground surface and protected from rain by a big, flat stone, placed on some smaller one arranged around the trap in order to leave the entrance accessible. The trap was filled with a non-toxic solution of alcohol 96%, wine-vinegar and water (1:3:6; alcohol is not mandatory, but could allow a better preservation of organisms collected at the beginning of the trap activity.) with salt and 1–2 drops of soap to catch and preserve the animals during the sampling period (Gobbi, 2020 modified adding alcohol for better preserving springtails); sampling period: 20 July 2022 – 20 August 2022.

### 2.3. Data analysis

Specimens were identified to species or genus level with taxonomic key reported in Gisin (1960), Bretfeld (1999), Potapov (2001), Thibaud et al. (2004), Jordana (2012) and Mateos (2011); specifically, identification was performed at genus level if the specimens were too few (or at the juvenile stage) for a finer taxonomic identification. Each species was classified as euedaphic/hemiedaphic/epiedaphic/atmobiotic for the analysis of functional composition according to Potapov et al. (2016) and then euedaphic/hemiedaphic were grouped into “soil springtails” and epiedaphic/atmobiotic into “ground-dwelling springtails”, in order to test if the three methods sample distinct communities from a functional point of view.

The average number of species collected per sampling point by each method and by each possible combination of different methods was compared with ANOVA and post-hoc Tukey test.

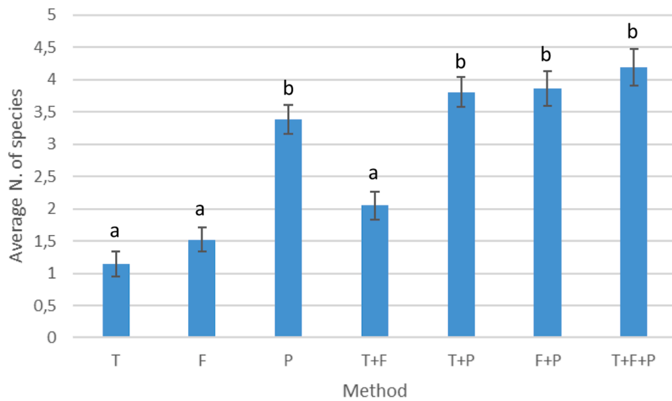


**Fig. 3.** Average number of species collected by each sampling method and each combination of methods. T = Tullgren funnels, F=flotation method, P= pitfall trapping. Error bars indicate standard error. Letters a-b indicate homogeneous groups assessed by Tukey's post hoc test.

The number of specimens collected by Tullgren funnels and by Flotation method can be directly compared as both methods extract the springtail assemblage from a fixed volume of soil (200 ml in the present research). Pitfalls, on the other hand, operate along a specific time interval (1 month in the present research) on an undefined surface, which depends from the specific mobility of each species; the absolute number of collected specimen cannot thus be compared with those obtained by the previous methods. For this reason the number of specimens was compared with ANOVA and Tukey test only among Tullgren funnels, Flotation method and the combination of the two. We compared both the overall number of specimens and that of each sampled species separately.

The potential effect of different sampling methods on the abundance of each species was evaluated by using N-mixture models. Again, for the reasons explained above the effects on abundance data were tested only for Tullgren funnels and Flotation method. N-mixture models are commonly used to take into account factors affecting the detection process, integrating their effects with that of variables influencing the state process. In this case, the latter were not considered and set to a constant. The number of sampled individuals for a single taxon found during a survey was taken as the dependent variable in abundance models, whereas the sampling method was entered as factor driving the detection process. First, all models were fitted with a Poisson distribution. Then, we performed a validation test by means of a goodness-of-fit (GoF) test based on 99 simulations. From this set of simulation, we derived the GoF's P and c-hat values, and considered models as validated when $P > 0.05$ and c-hat $< 1.5$. Given that all models were not validated, a negative binomial error structure was adopted instead of the Poisson distribution, and the validation test was run again and led to validate all models (with the partial exception of *Entomobrya*, *Heterosminthutus* and *Isotomurus*, which resulted in a c-hat value between 1.52 and 1.7, and the exception of *Proisotoma* and *Pseudisotoma*, which showed a c-hat value between 2.2 and 2.4). The models were built in R (R Development Core Team, 2020), by means of the packages 'unmarked' (Fiske and Chandler, 2011), MuMin (Bartoń, 2020) and 'AICcmodavg' (Mazerolle, 2020).

Then, we assessed whether the effect of the sampling method was statistically supported or not by comparing the Akaike's information criterion corrected for small sample size (AICc) for models respectively including as factors affecting the detection process i) a constant, ii) the sampling method. If the AICc of the model including the sampling method was lower than the other one, we considered the effect of the sampling method on the outcome of springtail collection as supported.

The incidence coverage estimator (ICE) was used to estimate potential true richness; ICE estimates the overall number of species that may live at one site, based on the observed number of species and the frequency of their occurrence, and it is particularly recommended when small grain pitfall trapping design was applied (Hortal et al., 2006; Colwell, 2013).

In order to take into account the effect of non-comparable sampling methods on the absolute number of collected specimens, we calculated the relative abundance of each species for each sampling method (dividing the absolute abundance of each species by the total number of specimens collected by the method). Relative abundance values were used to assess the dominant species of the overall sampled community avoiding the bias due to the higher number of specimens inherently collected by a specific method. A non-metric multidimensional scaling (NMDS) with Chord distance based on relative abundance was performed to visually represent the differences between the communities sampled with the different methods.

## 3. Results

A total of 11 species was detected; of these, nine were sampled with Tullgren funnels, eight with pitfall trapping and seven with flotation method (Fig. 3, Supplementary Table 1). Of the 1083 specimens globally collected, 763 were sampled by pitfall trapping, 236 by flotation method and 84 by Tullgren funnels (Fig. 3, Supplementary Table 1). Considering the relative abundances of each species collected by each method (Supplementary Table S1-S2), the three most abundant species were *Pachyotoma crassicauda*, *Orchesella* cf. *alticola* and *Vertagopus* sp n.

Pitfall trapping sampled a significantly higher average number of species in each sampling point (3.38) than the other methods alone (ANOVA and post-hoc Tukey test, $p < 0.001$). The highest average number of species in each sampling point (4.19) was collected by the three methods together, followed by pitfall trapping combined with flotation method and pitfall trapping combined with Tullgren funnels (3.85 and 3.80 species, respectively); all these values were not

**Table 1**
Summary of model results, validation (GoF P: P-value of the goodness-of-fit test; c-hat: value of the c-hat statistic; see text for details), and AICc, In bold, values lower than the constant-only model, which indicate support for the effect of the sampling method on the number of individuals/taxa found during the sampling. For the two methods compared, Tullgren funnels and Flotation method, the estimated coefficient plus its standard error is reported, representing the method's effect compared to the intercept. Gray background indicated models not validated (see text for details)). Legend: F=springtail functional category: g= ground-dwelling, s = soil springtail.

| Taxon | F | Tullgren funnels | Floation method | GoF *P* | c-hat | AICc const. | AICc method |
|---|---|---|---|---|---|---|---|
| *Vertagopus* sp.n | g | -4.25 ± 0.654 | -1.93 ± 0.655 | 0.43 | 0.9 | 214.03 | **139.35** |
| *Entomobrya* sp | g | 2.89 ± 574 | -9.75 ± 131 | 0.01 | 1.55 | 16.89 | 19.59 |
| *Heterosminthutus diffusus* (Gisin, 1962) | g | -9.75 ± 131 | 2.89 ± 574 | 0.33 | 1.7 | 16.89 | 18.59 |
| *Hypogastrura* sp. | g | 2.39 ± 153 | -10.60 ± 136 | 0.37 | 1.14 | 23.59 | 23.91 |
| *Isotomurus palliceps* (Uzel, 1891) | g | 11.8 ± 369 | 11.8 ± 369 | 0.37 | 1.66 | 15.50 | 18.59 |
| *Lepidocyrtus instratus* Handschin, 1924 | g | 2.39 ± 153 | -10.60 ± 136 | 0.31 | 1.10 | 23.59 | 23.91 |
| *Orchesella* cf. *alticola* Uzel, 1891 | g | -10.60 ± 136 | 2.39 ± 153 | 0.40 | 1.13 | 23.59 | 23.91 |
| *Pachyotoma crassicauda* (Tullberg, 1871) | g | -3.16 ± 0.244 | -1.94 ± 0.228 | 0.19 | 1.39 | 289.48 | **238.89** |
| *Parisotoma* sp. | s | 0.615 ± 1.26 | 0.615 ± 1.26 | 0.63 | 0.93 | 36.90 | 39.98 |
| *Proisotoma* sp. | g | -2.43 ± 1.06 | -5.15 ± 1.38 | 0.08 | 2.24 | 59.37 | **48.54** |
| *Pseudisotoma sensibilis* (Tullberg, 1876) | g | 2.72 ± 89.4 | -10.23 ± 66.0 | 0.13 | 2.41 | 30.31 | **24.91** |

**Table 2**
Comparison between the observed species richness (count data) and the estimated species richness (incidence-based non-parametric diversity estimator) for each method.

| Total number of species observed | Sampling method | Observed species for method | Estimated species (ICE Index) | % of sampled species (observed vs estimated) |
|---|---|---|---|---|
| 11 species | Tullgren funnels | 9 | 12 | 75 |
| | Flotation method | 7 | 13 | 54 |
| | Pitfall trapping | 8 | 10 | 80 |

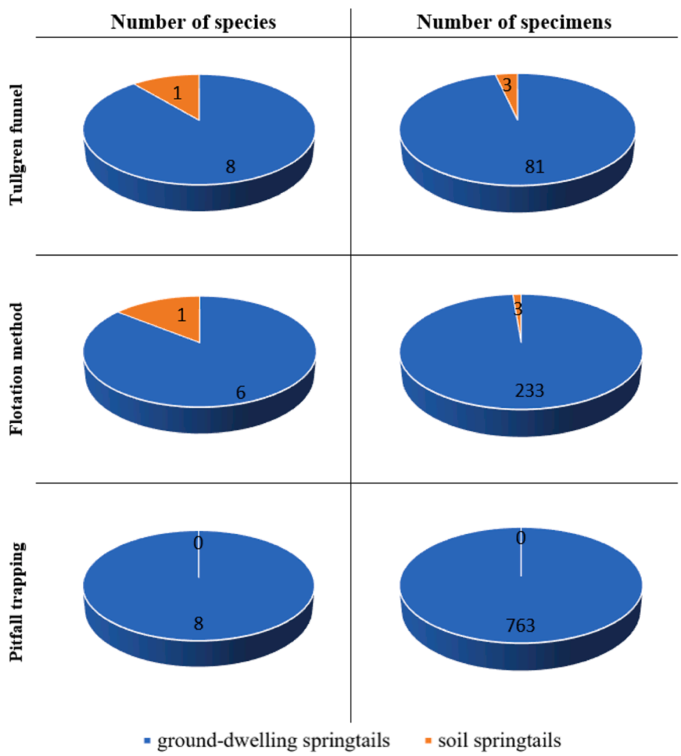


**Fig. 4.** Composition of the community sampled with the three different sampling methods. The absolute number of species and specimens found for each functional category are reported.

statistically different from each other and from that of pitfall trapping alone. The combination of flotation method and Tullgren funnel collected on average only 2.04 species for each sampling point; this value was not statistically different from those from these two methods alone (1.14 and 1,52 species for Tullgren funnel and for floating, respectively; ANOVA and post-hoc Tukey test) (Fig. 3, Supplementary Table S3).

Tullgren funnels and flotation method collected on average 3.95 and 11.19 individuals per sampling point, respectively, while their combination collected 15.14 individuals; the only significant difference was observed between Tullgren funnels and the combination of the two methods (ANOVA and post-hoc Tukey test, $p = 0.002$). Considering the single species, the same pattern was observed for *Vertagopus* sp. and for *Pachyotoma crassicauda* (ANOVA and post-hoc Tukey test, $p = 0.049$ and 0.045, respectively), while the other species did not show significant differences (Supplementary Table S4).

All the N-mixture models were validated (with Poisson or negative binomial distribution) with the only exceptions of *Proisotoma* sp. and *Pseudoisotoma sensibilis*, for which c-hat value was too large, and *Entomobrya* sp., for which P value was lower than 0.05 (Table 1). High standard error associated to some estimates were likely due to the large variations in the number of sampled individuals, coupled with the low sample size. The effect of the sampling method was statistically supported for *Vertagopus* sp. and *Pachyotoma crassicauda* (Table 1), which were sampled in higher quantity by the flotation method.

*Vertagopus* sp. n., a new cryophilic species currently under description by the authors and particularly linked to supraglacial debris, was collected mainly by flotation method and secondarily by pitfall trapping with respect to Tullgren funnels (Table 1, Supplementary Table S1-S2). On the other hand, *Orchesella* cf. *alticola* and, to a lesser extent, *Pachyotoma crassicauda* were collected more by pitfall trapping and secondarily by flotation method with respect to Tullgren funnels (Table 1). Species exclusively sampled by Tullgren funnels were the species *Entomobrya* sp. (1 specimen) and *Hypogastrura* sp. (2 specimens) but the effect of the sampling method on these species was not statistically supported (Table 1), because of the very low sample size; the other two methods did not sample exclusive species. On the other hand, only pitfall trapping and flotation method detected all the three dominant species, while Tullgren funnels did not collect *Orchesella* cf. *alticola*.

The incidence coverage estimator (ICE index; Table 2) showed that the estimated number of species detectable by Tullgren funnels and flotation method are similar (respectively 12 and 13), while for pitfall trapping the number is lower, 10. On the other hand, the three methods sampled 75 %, 54 % and 80 % of the estimated species, respectively (Table 2).

From the functional point of view, the springtail community is mainly composed by ground-dwelling species, and, to a much lesser extent, by soil species (Fig. 4). However, the number of species and individuals belonging to each functional category change in relation to the sampling method used; in particular, pitfall trapping did not sample soil springtails at all (Fig. 4).

The communities collected by the different methods in the same sampling point are not close to each other in the NMDS plot, while those obtained by the same method tend to cluster together (Supplementary Fig. S3).

## 4. Discussion

### 4.1. Comparison of methods: Sampling efficiency

In the investigated glacial species-poor terrain, no method was able to collect the whole community.

This result indicates that no single method stands out as the most efficient for the complete characterization of the springtail community. A combination of different methods should be carefully evaluated in order to maximize the obtained information.

Pitfall trapping collected a higher average number of species in each sampling point than the other methods. Pitfall trapping is useful in many different habitats - with some differences in efficiency in relation to bedrock, microclimatic and edaphic parameters (López and Oromí, 2010; Mock et al., 2015; Nitzu et al., 2018), and is often the sole method used for characterizing ground-active arthropods (Brown and Matthews, 2016), even in proglacial habitats (Valle et al., 2022b). However, this method is efficient for collecting ground-dwelling organisms, such as atmobiotic and epiedaphic springtails, and its use alone could overlook soil springtails (Siewers et al., 2014; Hohbein and Conway, 2018) as observed in our research, where the sole hemiedaphic species found – *Parisotoma* sp. - was not detected by this method. It is interesting to observe that, in our study system, pitfall trapping gave the highest observed/estimated species ratio (80%) mainly due to the low number of the estimates species rather than to the high number of the observed ones. These data suggest that pitfall trapping is highly efficient in sampling a more limited set of potential species. These considerations confirm that an efficient combination could consist of the addition, to pitfall trapping, of a method able to collect soil springtails (Querner and Bruckner, 2010; Nsengimana et al., 2017), as already experimented in proglacial sites by Valle et al. (2022a). Coherently, our results showed that the combination of Pitfall trapping with any other method further increased, although not significantly, the already high average number of species detected, as well as the detected functional types.

Both Tullgren funnels and flotation method are designed to extract the springtail community from a given volume of soil and provided similar results concerning the average number of species and the functional composition of the detected springtail community. The overall overlapping of the two methods is confirmed by the fact that their combination did not increase significantly the detection performance of the two methods alone, and was the least efficient combination of methods. Tullgren funnels detected a higher number of species, and it was the only method that sampled two exclusive species. On the other hand, it failed in collecting the abundant taxon *Orchesella* cf. *alticola*.

The choice of the best method to be associated to pitfall trapping is thus not straightforward. The higher number of species collected by Tullgren funnels could be a decisive factor, as the detection of the highest possible number of species is definitely a goal to be achieved. However, all the statistical analyses did not give statistical support to any different performance between the two methods. As the two methods are supposed to collect exactly the same amount of specimens (since they should extract all the specimens occurring in the same volume of soil), we believe that the much higher number of specimens found through the flotation indicates its greater ability to extract springtails from mineral soil, as already suggested by Marshall et al. (1994). This being the case, the two exclusive species detected by Tullgren funnels (*Entomobrya* sp. and *Hypogastrura* sp.) could have been collected stochastically, as suggested by their very low frequency and abundance (they both occurred once with 1 and 2 specimens, respectively) and the fact that the two methods do not significatively differ for the collected average number of species. A further advantage of flotation method is that it does not collect only actively moving organisms, but, potentially, all the organisms occurring in the soil samples, including dead individuals, providing also complete life cycle data for many species (Kethley, 1991).

Given these consideration, we recommend the combination between pitfall trapping and a method able to collect soil springtails; among these, flotation method is, in our opinion, the most appropriate for mineral soil in glacial environment, although the low observed/estimated species ratio (54 %) suggests that this method could require a higher number of sampling points, which would probably allow the detection of the rare missing species. Our data indicated a marked higher efficiency of flotation method in detecting the cryophilic species *Vertagopus* sp. n. This suggests that floating is recommendable when the target is monitoring the occurrence and population size of cryophilic species. On the other hand, flotation method cannot be easily applied in species-rich, organic soils (Kethley, 1991), therefore it could be not adequate for sampling plans that include mature soils, like those occurring on areas ice-free since more than 100 years (Brambilla and Gobbi, 2014). For these stable and mature habitats, Tullgren is the most efficient method for collecting springtails, being used for comparing data even at global scale (Potapov et a, 2022). This means that for the study of the primary succession of springtail communities on glacier forelands different approaches should be taken into account when dealing with pioneer stages, such as those discussed in this paper, and late-successional ones, usually occurring on terrains dating back to the Little Ice Age (c.1850). In Norwegian proglacial sites Hågvar (2010) extracted soil fauna with Tullgren funnels (modified by Macfadyen, 1961); however, that Scandinavian glacier foreland is less sloping, more sandy-silty and moist (as suggested by the vegetation dominated by *Salix herbacea;* Landolt, 1977), thus quite different from Alpine glacial soils, usually sloping and coarse.

However, flotation method probably requires a higher number of sampling points or temporal replicates to collect all the potentially detectable species. This weakness could be solved by applying this method two times: the first one during the first field session after the setting of the pitfall traps, and the second one when the researchers come back to the field to collect the traps. Furthermore, if the scientific study plans to compare mineral glacial soils with more organic soils, Tullgren funnels rather than flotation method should be applied (e.g. Haybach, 1972); this method, however, would probably underestimate the densities of springtails in mineral soils.

### *4.2. Comparison of methods: Conservation issues*

The collection of a large number of specimens is usually necessary to allow a detailed taxonomic identification and to estimate the relative abundance of the detected species. However, conservation issues arise when dealing with severely threatened habitats and species (Lencioni and Gobbi, 2021). This could be the case of cryophilic species belonging to the genera *Vertagopus* and *Desoria* (Potapov, 2001) and of stenoendemic species strictly linked to glacial environment on rapidly shrinking glaciers, such as the case of the recently described cryophilic springtail *Desoria calderonis* (Valle et al., 2021). In this case, the choice of the sampling method should take into account the most favorable species/specimens ratio, to maximize the information minimizing the impact on the population; such criterion is met by flotation method and by Tullgren funnels.

### *4.3. Other considerations on environmental heterogeneity*

In our sampling no euedaphic springtails (i.e., adapted to live in hypogean habitat or to deep layer of the litter; Potapov et al., 2016) has been collected. This observation is plausible in relation to the time since deglaciation of the sampled lithosols. Probably less mobile life forms, like the euedaphic ones, need more time to colonize young terrains because they show demanding requirements in terms of soil organic matter content (Potapov et al., 2016). Since mosses and cushion vascular plants could facilitate microarthropod communities in harsh habitats (e.g. Ľuptáčik et al., 2021; Coulson and Midgley, 2012), we could not exclude that these cushions - few and sparse and excluded by our samples because of the stochastic selection of sampling points - could locally host euedaphic species linked to more stable and mature micro-habitat, as previously observed on other glacier forelands (e.g., Forni Glacier, Italian Alps, close to Sforzellina glacier; B.V. personal observation). For this reason, a useful integrative qualitative sampling could include the selection of moss cushions for the Tullgren funnels.

In this work we only considered methods applicable at ground level on soils with few interstitial space. Where the stony debris is coarse, deep interstices and subsoil fissures could host a peculiar fauna (Mesovoid Shallow Substratum; Baquero and Jordana, 2022). In Alpine high-elevation environments this condition can be observed on rock glaciers (Gobbi et al., 2014) or in coarse scree slopes. In order to collect fauna in the deep substrate, subterranean sampling devices (SSDs) could be a valid method (Baquero et al., 2017; Jureková et al., 2021).

### *4.4. General remarks on sampling and storage efforts*

The low accessibility of Alpine glaciers and the importance in optimizing the sampling effort are key to monitoring in glaciers and other high-elevation environments. In these terms, pitfall trapping critical point is the need for preservative solution, that constitutes a significant weight to be transported; a similar problem occurs with flotation method, because of the large quantities of water needed; however, water is often available on the field from the glacial streams. In addition, the use of pitfall trapping needs to plan at least two sampling sessions, for activating and emptying the traps, while flotation method and Tullgren funnels could be applied in a single session.

Concerning sample storage, pitfall trapping and flotation methods do not require special precautions, since it is possible on the field to directly

**Table 3**
The answers of the three methods to different methodological issues are synthetized. Information comes from our data; if not, the reference is reported.

| | | | | | | | Efficiency and functional composition of collected community | |
|---|---|---|---|---|---|---|---|---|
| | Field work effort | Lab work effort (not considering taxonomic identification) | Number of species collected | Number of specimens collected | Dominant species collected | Life-cycle data | Inorganic soils (including supraglacial debris) | Organic soils |
| **Tullgren funnels** | Single visit of sampling site, fast sampling, but transport of heavy and delicate soil samples back to the laboratory | Many days necessary for processing soil samples | 9 | 84 | 2/3 | - | - | Efficient for collecting soil springtails in organic soils (Marshall et al. 1994) |
| **Flotation method** | Single visit of sampling site, time-consuming work on the field for manually collecting specimens, but light samples to be carried at laboratory | - | 7 | 236 | 3/3 | It samples also less mobile organisms, like juvenile (Kethley, 1991) | Efficient for collecting soil springtails in lithosols | - |
| **Pitfall trapping** | Double visit of sampling site, time-consuming work on the field for activating the traps. Light samples to be carried at laboratory | - | 8 | 763 | 3/3 | - | Efficient for collecting ground-dwelling, mobile springtails in both type of soil | |

transfer the specimens collected in tubes filled with alcohol (96 %). Tullgren funnels necessarily need the samples to be carried out as soon as possible (Edwards, 1991) to prevent the sediment from moving and shredding the specimens. Tullgren funnels also request further day-works in laboratory for quickly process the samples before the organisms die.

## 5. Conclusion

Our data suggest that in lithosols of Alpine glacial habitat the best solution for optimizing quantitatively sampling of springtail community is to combine pitfall trapping with a method that extract soil springtails; we suggest to use flotation method, based on sampling and storage effort, on our data and on bibliographic consideration, for analysing the springtail community in incoherent lithosols (Table 3). For sampling plans that includes also mature soils, pitfall trapping and Tullgren funnels are the best combination.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

data can be found in the main text.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.pedobi.2023.150914.

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