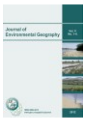
*Journal of Environmental Geography* 16 (1–4), 46–54.

DOI: 10.14232/jengeo-2023-44683
ISSN 2060-467X



# AUTOMATIC DROUGHT TOLERANCE MEASUREMENT OF THE SOIL-LIVING MICROARTHROPOD, *FOLSOMIA CANDIDA*

**László Sipőcz[1*], András Ittzés[2], Miklós Dombos[1]**

[1]Institute for Soil Sciences, Centre for Agricultural Research, ELKH, Herman Ottó út 15., 1022 Budapest, Hungary
[2]Hungarian University of Agricultural and Life Sciences, Institute of Mathematics and Basic Science,
Villányi út 29-43., 1118 Budapest, Hungary
*Corresponding author: sipocz.laszlo@atk.hu

Research article, received 15 May 2023, accepted 19 July 2023

## Abstract

Soil is a complex habitat where microarthropods, such as mites (*Acari*) and springtails (*Collembola*) species occur in high number and species diversity. Microarthropods play an essential role in organic matter decomposition and provide an important ecosystem service in soil. The soil-dwelling microarthropods are sensitive to environmental changes; therefore, their ecological characteristics are used to evaluate soil conditions. In modern environmental ecology, several species are involved in assessing the ecological consequences of drought periods. The growth rate is a standard sublethal parameter by which the body size of individuals is measured. Extracting microarthropods from the soil is difficult and time-consuming, requiring a high amount of human resources. Only a few samples can be processed due to laboratory limitations and high costs. However, nowadays the rapidly developing artificial intelligence (AI) technologies promise new opportunities in many research areas.

Data on soil-dwelling microarthropods could be collected quickly and automatically using our new digital soil extractor device, the Edapholog, equipped with image analysis based on AI. This device recognizes living individuals, classifies them, and measures their body length automatically. Using this system, the growth and reproductive success of various species in the same experimental culture could be rapidly and simultaneously monitored. In this study, we aimed to analyse the applicability of the Edapholog for measuring the body size of *Collembola* species and *Folsomia candida* through a set of drought tolerance tests with three soil moisture treatment levels. Moisture content was set based on the maximum water holding capacity ($W_{max}$) of the soil applied. The three levels were set to $W_{max}$:35%, 40%, and 50%. Furthermore, we aimed to test the reliability of the detection and recognition of the species and the accuracy and reliability of the automatic body size measurement of individuals.

Significant correlation (r= 0.94) exists between the automatically and manually measured body sizes. Although the different soil moisture treatments did not show marked differences in the collembolan body sizes between the moisture treatments, we found a significant difference in the reproduction rates between $W_{50}$ and the other two ($W_{35}$ and $W_{40}$) treated groups. The Edapholog can greatly contribute to quick and precise data extraction and can have vast applicability in environmental ecology.

**Keywords:** ecotoxicology, artificial intelligence, microarthropods, *Folsomia candida*, drought tolerance

## INTRODUCTION

The more is known about the ecological processes of the soil, its vulnerability becomes more apparent. Soil provides clean air, food, and water; therefore, the importance of its protection is evident (Pereira et al. 2018). The soil-dwelling mesofauna (mainly microarthropods) plays an essential role in the decomposition of plant organic matter and nutrient cycling in the upper layer of the soil (Briones 2014). Microarthropods (mainly mites and springtails) feed on soil-dwelling fungi and other microbes and play a top-down regulatory role in the decomposing food chains (Coleman et al. 2017). The mesofauna is present with a huge number of individuals (~$10^5$/$m^2$) and species in many types of habitats. They also influence the nutrient exchange processes of the soil. So they can indirectly play a significant role in the quantitative regulation of the sequestration of atmospheric carbon dioxide (Filser 2002).

Soil-dwelling microarthropods react sensitively to various soil conditions and pollution; therefore, they are potential bioindicators (Brussaard et al. 2007, Briones 2014). Generally, the more intensive the soil life, the healthier the soil is (Menta et al. 2018). Extreme weather events resulting from global climate change have become one of the most significant problems recently (Steffen et al. 2007). Adapting to these events and mitigating their effects is necessary (Kardol et al. 2011, Makkonen et al. 2011, Meehan et al. 2020). The biodiversity and organic matter of soil are highly dependent on the soil moisture level. Drought periods seriously impact the diversity of species living in the soil and threaten essential ecosystem services. Without soil-dwelling microarthropods, organic matter formation may decrease, atmospheric carbon dioxide sequestration levels may slow down, and the water retention capacity of soils may also deteriorate (Bardgett 2005, Briones 2014). Therefore, there is an increasing need to study the influence of drought on soil-dwelling mesofauna.

However, examining soil-dwelling microarthropods is quite complicated and labour-intensive. Still, today's rapidly developing information technology (IT) solutions promise new possibilities to solve these difficulties. Artificial intelligence (AI) is increasingly popular in various research fields, thus it became popular among scientists, and it could facilitate data collection even by involving lay citizens (Silvertown 2009, Bilyk et al. 2020). The most popular AI applications in ecology are based on sound and image processing (Mallard et al. 2013, Liu et al. 2018, Christin et al. 2019, Flórián et al. 2020, Høye et al. 2021, Laursen et al. 2021, Sys et al. 2022).

A new device has been developed by the soil zoological research group at the Centre for Agricultural Research – Institute for Soil Sciences (ELKH) and Edaphone Ltd, using image recognition and AI-based image analysis methods. This device substantially differs from those previous Edapholog models that used an opto-electronic ring and CCD camera and functioned as a traditional pitfall trap. Those prototypes measured soil-dwelling mesofauna's activity under field conditions (Dombos et al. 2017, Gedeon et al. 2017, Balla et al. 2020). The new Edapholog2022 model is an automatic measuring device that detects microarthropods, takes photos, and measures their body sizes. After that process, it blows the detected individuals off. Therefore, Edapholog can also be suitable for the measurements of eco-toxicological tests. For eco-toxicological tests, some ISO standards exist (ISO, 1999; ISO, 2006; ISO 2011), and some innovative tools and programs have been developed. For example, the CollScope developed for eco-toxicological testing, which measures the body sizes of springtails (Bánszegi et al. 2014). The digital soil extractor based on AI image processing may help replace human manual work. In addition, the exact detection time can be used as a new dimension, which can play an important role in drought tolerance studies of mesofauna.

We performed a laboratory microcosm experiment in which *Folsomia candida* Willem 1902 individuals were kept under different soil moisture conditions. The Edapholog digital soil extractor examined their sublethal parameters like body size. *F. candida* is a popular springtail species used in eco-toxicological tests. Edapholog is produced by Edaphone Ltd., and we tested its usability for eco-toxicological tests.

The following research questions were answered: 1) Is there a difference between the body sizes of animals kept under different soil moisture conditions? 2) Can the difference in body sizes be detected with the new device Edapholog? and 3) What is the accuracy of the measurement?

## MATERIALS AND METHODS

### *Test animal and materials*

The drought tolerance test was conducted on a synchronized population of *F. candida* Willem 1902 (*Collembola*) individuals, often used in eco-toxicological tests (Bayley et al. 2001, Lock and Janssen 2002, Hilligsøe and Holmstrup 2003, Sørensen and Holmstrup 2005). The culture was kept in continuous darkness at 20±°C in a 15 cm diameter plastic tube with a closed lid. At the bottom of the tube was a 1.5 cm thick moistened plaster of Paris mixed with activated carbon. The gypsum to activated carbon ratio was 1:8 by volume. The culture was fed once a week *ad libitum*. The moisture content of the plaster was also adjusted once a week based on the initial weight of the sample holders. According to the plaster's maximum water absorption capacity, it was always restored to 100% hydration every week.

Synchronization of populations was carried out in a cylindrical box (diameter of 20 cm) with 2 cm of 1:8 mass volume carbon plaster on the bottom. The maximum saturation of the charcoal plaster was achieved by the following: after drying, water was added to the plaster and incubated for 10 minutes. Then, water was poured off, and the weight of the container was measured. This weight was always set back with a flask with a spray head. The synchronization was performed by keeping 200-300 adult individuals taken from *Folsomia candida* culture together in this cylindrical box for three days. The *Collembola* laid enough eggs during this time to start the experiment (OECD, 2009).

The experimental medium was commercially available general potting soil. The potting soil was sieved according to soil aggregate sizes, then the aggregates within the size range of 2-5 mm were selected to ensure undisturbed extraction of the animals. Since potting soils often contain soil-dwelling microarthropods, defaunation had to be carried out, thus, the sieved soil was heat-treated at 108°C for 72 hours.

### *Experimental design*

The experiment was carried out at a constant temperature (20±1°C) with 12-hour-long dark and light periods. The lighting was ensured with cold white LED lamps, producing a light intensity of 580-600 Lux. The dried soil (20 g) was measured into a 120 ml plastic sample holder (diameter: 5 cm). The moisture content of the samples was set according to the soil's maximum water-holding capacity ($W_{max}$).

To calculate the $W_{max}$, air-dried potting soil samples were taken into three special metal sample tubes. At the bottom of the sample tubes, a moisture-permeable nylon fibre screen fabric was attached. These samples were gradually saturated with water for a week. The weight of the three sample holders containing potting soil saturated with water was measured and then placed on a 6 cm thick layer of sand for one hour. The unnecessary, unbound water leaked out. The samples were dried in a drying oven at 108 °C for 48 hours, and then the weight of each sample holder was re-recorded. The difference in the mass of the potting soil saturated with water and the dried potting soil was the $W_{max}$. The three figures obtained this way were used during the subsequent moisture adjustment (OECD, 2009).

*F. candida* is a so-called edaphic species, thus, it lives in the deeper soil layers, in the cracks and pore spaces of the soil, so it is not advisable to set a moisture value higher than 60% of the $W_{max}$. For this reason, 50% of Wmax was set as the highest soil moisture value. The soils received three moisture treatments, 35% ($W_{35}$), 40% ($W_{40}$), and 50% ($W_{50}$) of the $W_{max}$. $W_{35}$ corresponds to

14.95 V/V%. Similar conditions evolved during the drought of 2022, as the average soil moisture in August at a depth of 10 cm was 15.16 V/V% according to the measurement station near Kiskunfélegyháza in Hungary ("Aszálymonitoring" 2023).

The soil moisture levels were set in every plastic sample holder one week before the experiment started.

Ten individuals of 9-12-day-old *F. candida* were placed in each plastic sample holder. The duration of the experiment was four weeks. The response variables were the body length of the individuals measured manually and automatically. The moisture contents were adjusted in a newly equipped extractable sample holder with three repetitions. The sample holder tube was closed with a screw cap at the top and a rubber cap at the bottom to increase the sealing effectiveness. The rim of the rubber cap was further secured with insulating tape. Even so, an average of 0.12 g of water evaporated weekly, continuously replenished during the inspections. The plastic sample holder was also equipped with a 2x2 mm mesh at the bottom, to which a sealing rubber cap was attached. The moisture content of the samples was checked three times a week. The necessary moisture replenishment was based on regular weighing. The test animals were fed with baker's yeast ad libitum three times a week. The samples were placed in a 3x3 grid, and the placement was randomly changed at each check to reduce possible distorting effects.

Extraction of animals from the sample holder tube was done with a Berlese-Tullgren funnel. By removing the caps from the bottom, the animals left the samples without disturbance along the vertical gradient of soil drying. The extraction time was two days because of the small amount of soil, which dried quickly.

*Automatic detection of soil microarthropods*

In this experiment, the new equipment prototyped by Edaphone Ltd. (Esztergom, Hungary) was used to extract collembolan individuals from the microcosms. This soil extractor follows the operating principle of the traditional Berlese-Tullgren funnel (Crossley and Blair, 1991). A soil sample – without the plastic cap – was placed on a sieve box, and a vertical moisture gradient triggered microarthropods to escape. They fall through a mosquito mesh (2x2mm) and entered the sample holder through a funnel (Fig. 1). During soil extraction, soil particles also tend to fall into the sample container. To prevent this, we used a 3 cm layer of gravel.

Contrary to the traditional soil extractors where microarthropods were captured and killed in a sample container, in this new equipment, microarthropods entered into a plate where they were video recorded. For video recording, the device houses a high-resolution camera (IMX477R stacked, back-illuminated sensor, 12.3 megapixels) and a lens (Machine vision FA lens, 10 MP, 51.5 mm 1/ 1 /8", C-mount). An image analysis procedure occured on each picture in real-time during video recording. A detailed description of this prototype will be published in a forthcoming paper. After detection, the microarthropod individuals were removed by an automatic blow-off pump into a sample container containing a preservation liquid (70% ethanol + Nonit: 60% dioctyl sulfosuccinate Na-salt). Thereby, the biological sample could be used for further analysis.

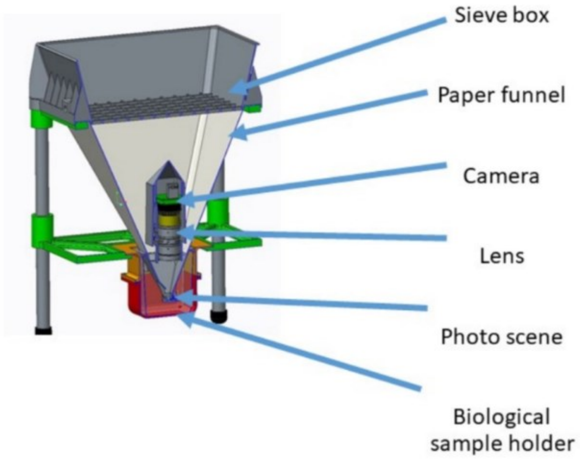


*Fig.1* The new Edapholog device is based on the conventional soil extractor. The soil invertebrates, triggered by drought, move downwards and fall through the sieve box and the funnel. Photographing of the individuals (detection and identification) occurs in real-time under the funnel where individuals fall.

*Automatic measurement of body length*

The software developed by Edaphone Ltd. recognizes the animal by pixel extraction calculated by deviation from the background. In the body size estimation process, the first step is to detect the animal. Then, the program frames and defines the boundaries of the animal. Contour creation is a multi-step process. First, the image is grayscaled, then by setting a threshold value, it is divided into only black and white pixels, and finally, the contour of the detected animal is highlighted. The body size will be the length of the segment connecting the x and y maximum of the contour (Fig. 2).

Twenty automatically measured body size data were obtained for every detected collembolan individual. Based on preliminary test measurements, we used the largest measured body length data for *Folsomia candida*. The reason for using the longest body length data is that during their movement, the animals can adopt body positions (turning, taking a defensive position), leading to smaller body lengths measured (Fig. 3). Registering the time of each measurement was also assigned to each animal as a unique identifier.

Animals were also measured manually by using the image obtained from the Edapholog device (Fig. 4). The body lengths were measured with the ImageJ software by drawing a straight line between the top of the insect's head and the end of its abdomen (Ninon et al. 2019).

At the beginning of the experiment, ten animals were placed in each sample holder, from which we calculated the reproduction rate (offspring/adult individual).

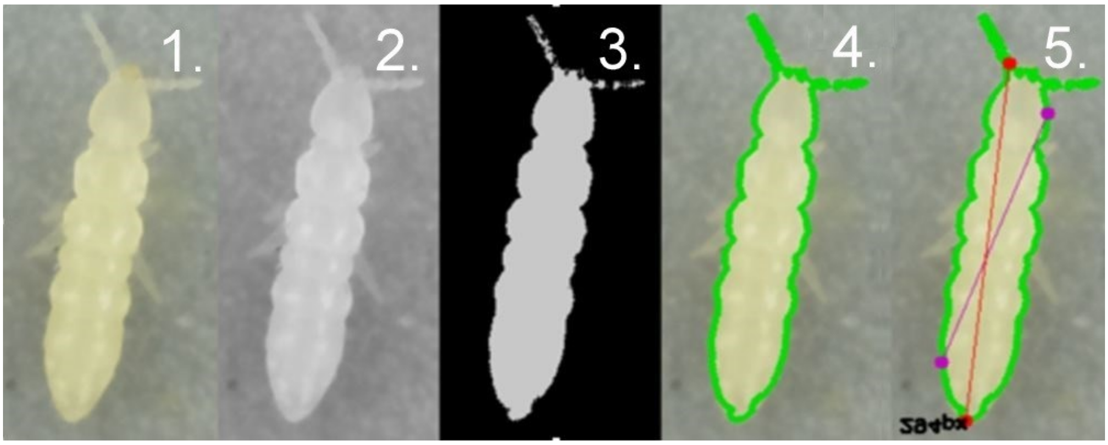
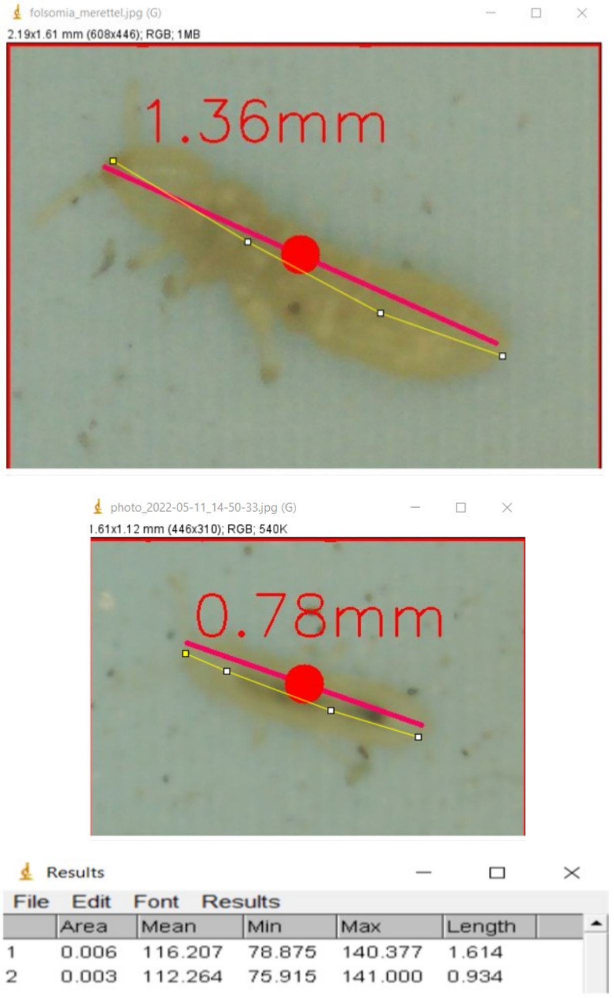


*Fig.2* Photo transformations for body size estimation.
1: animal framing and clipping, 2: turning the photo into grayscale, 3: splitting the image into black and white pixels, 4: drawing contours, 5: determining the animal's body length, based on maxima in the x and y directions.

### *Statistical analysis*

Body length values and the reproduction rates of the three different moisture treatments were analyzed using the one-way ANOVA. The dependent variable was the animal's body size (mm), and moisture treatment was the factor having three levels ($W_{35}$, $W_{40}$, $W_{50}$). Data are presented as mean ± standard deviation (SD). Collembolan body length values of the two measurement methods (manual and automatic) were analyzed with a Pearson correlation model. The large sample sizes (N>30) and the similarity of the variances justify the method's validity. Finally, a Bland-Altman plot was used to represent the differences between the two measurement methods and the mean values of the measured body length. The IBM SPSS v.27 (*IBPM SPSS Statistics for Windows 2020*) statistical program was used for all data analyses.

*Fig.3* Body positions that cause outliers during automatic body length measurements.



| | Area | Mean | Min | Max | Length |
|---|---|---|---|---|---|
| 1 | 0.006 | 116.207 | 78.875 | 140.377 | 1.614 |
| 2 | 0.003 | 112.264 | 75.915 | 141.000 | 0.934 |

*Fig.4* Manual and mechanical measurement of *Folsomia candida* individuals. The red line shows the result of the automatic measurement, while the yellow segmented line shows the manual observation.

## RESULTS

### *Drought tolerance experiment*

The body lengths of 729 individuals were manually measured and analyzed in the drought tolerance experiment (Fig. 5). The mean body length (using the manually measured data) in treatment $W_{35}$ (0.83±0.19 mm; N=139) was similar to the treatment $W_{40}$ (0.84±0.20 mm; N= 85). In the $W_{50}$ treatment, the mean body length (0.87±0.22, N=505) was slightly higher than in the lower soil moisture treatments. However, according to the results of the one-way ANOVA, the different soil moisture treatments have no significant effect on the body size of *F. candida* individuals (F (2;726)=1.681; p>0.05).

### *Reproductive success*

We found a considerable difference in reproduction success based on the number of sample elements of the treatment groups measured during moisture treatments, especially at $W_{50}$ (Fig. 6). While there is no significant difference between $W_{35}$ and $W_{40}$, $W_{50}$ significantly differs from these groups (Table 1).

### *Comparison of the manual and automatic measurement methods*

A close correlation was found between the results obtained by the manual and automatic measurement methods of individual body lengths. Analysing the data pairs of the manual measurement and the new automatic measurement, we got a strong relationship and a significant regression (Pearson correlation: r = 0.94; n = 730; p< 0.001). The average difference between the two measurement methods was 0.13±0.07 mm, which shows a systematic overestimation of body lengths by manual screening.

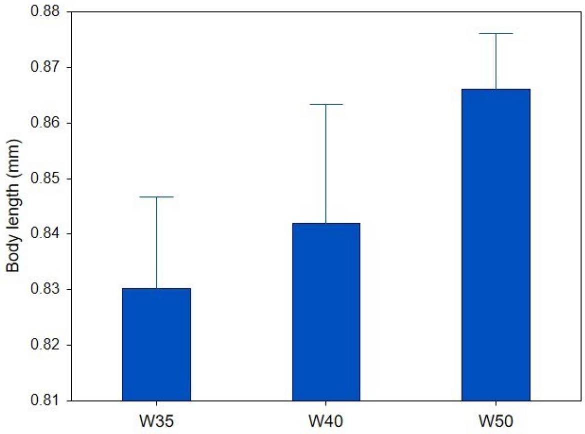


*Fig.5* Effects of soil moisture treatments on the body lengths of *Folsomia candida* based on manual measurements. $W_{35}$ is 35%, $W_{40}$ is 40%, and $W_{50}$ is 50% of the $W_{max}$ (Box depicts means, whiskers: standard errors, SE)

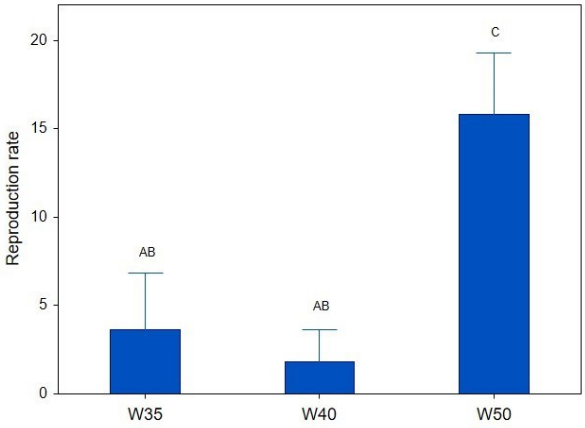


*Fig.6* Effects of soil moisture treatments on reproduction rates. $W_{35}$ is 35%, $W_{40}$ is 40%, and $W_{50}$ is 50% of the $W_{max}$ (Box depicts means, whiskers: standard errors, SE)

*Table 1* Results of ANOVA between the three moisture treatment levels on the reproduction rates (abbreviations: SS= sum of squares, MS= mean of squares)

| | Univariate Results for Each DV (reproduction) Sigma-restricted parameterization Effective hypothesis decomposition | | | | |
|---|---|---|---|---|---|
| Effect | Degree of Freedom | Reproduction | | | |
| | | SS | MS | F value | p value |
| Intercept | 1 | 453.69 | 453.69 | 53.9679 | 0.000326 |
| moisture | 2 | 348.08 | 174.04 | 20.7027 | 0.002028 |
| Error | 6 | 50.44 | 8.41 | | |
| Total | 8 | 398.52 | | | |

| | | Tukey HSD test; variable reproduction (reproduction) Approximate Probabilities for Post Hoc Tests Error: Between MS = 8.4067, df = 6.0000 | | |
|---|---|---|---|---|
| Cell No. | moisture | $W_{35}$ | $W_{40}$ | $W_{50}$ |
| | | 3.6333 | 1.8333 | 15.833 |
| 1 | $W_{35}$ | | 0.739079 | 0.005207 |
| 2 | $W_{40}$ | 0.73908 | | 0.002684 |
| 3 | $W_{50}$ | 0.00521 | 0.002684 | |

After transforming the automatic measurement data based on a fitted linear regression ($y = 1.026x + 0.1181$), we found no significant difference between the two measurement methods (Fig. 7).

The Bland-Altman plot can be used to compare data measured by different methods. It can also show whether one measurement method can be substituted for another. The two methods show minimal differences apart from a few outliers when comparing body length data obtained by manual and automatic measurements (Fig. 8). Overall, the average of the measurement differences depicted on the Bland-Altman plot remained in a narrow range. Hence, the difference between the results of the measurement methods is low.

## DISCUSSION

### *Drought tolerance*

Significant differences were found among the reproduction rates of the tested *Collembola* species, especially the $W_{50}$ setting showed conspicuously higher specimen numbers than the other treatments. The specimens had proper conditions before the experiment started, but then, they were exposed to sudden drought stress in the $W_{35}$ and $W_{40}$ treatments. The fauna may not have enough time to adapt to the low moisture conditions. Drastic and sudden drought rarely occurs in nature; therefore, the animals normally have time to adapt to the circumstances. The survival rate of springtails with time to adapt to drought or extreme temperatures is much higher than those exposed to sudden stress (Sjursen et al. 2001). However, *F. candida* can survive mild drought effects (Hilligsøe and Holmstrup 2003b), which may explain the reproductive success similarity between the treatments $W_{35}$ and $W_{40}$.

Unlike the reproduction rate, the drought tolerance test did not yield any significant changes in body size. In the experiment of Szabó et al., (2022; OECD, 2009), body sizes of *Folsomia candida* were also similar between the lower moisture treatments; however, these Collembolans got selenium treatments besides the drought stress. In our experiment, the survival and body size of ground-dwelling springtails were less affected by drought than the reproduction rate, similar to what has been shown for the dry mass of *F. candida* in the study of Wang et al. (2022).

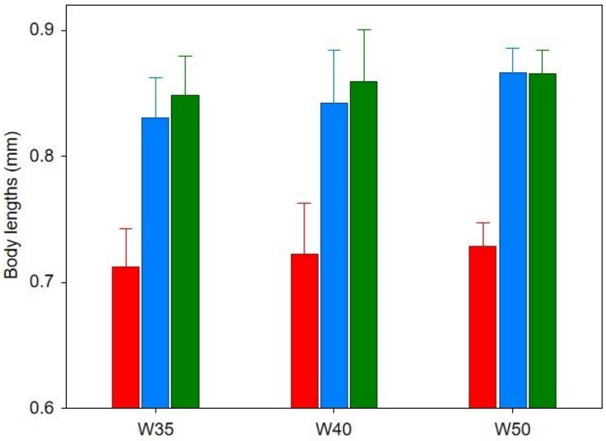


*Fig.7* Effects of soil moisture treatments on the body lengths of *Folsomia candida* based on manual (blue) and automatic measurements (red). The green columns depict the automatic measurements after transforming the data (by using linear regression ($y = 1.026x + 0.1181$).
$W_{35}$ is 35%, $W_{40}$ is 40%, and $W_{50}$ is 50% of the $W_{max}$
(Box depicts means, whiskers: standard errors, SE

Nowadays, sudden drought events are becoming increasingly severe, affecting soil health, and therefore some ecological services can be seriously damaged. By measuring the microarthropods found in the soil, we can infer the current state of each soil, therefore, it is important to analyse these organisms. Although no significant difference between the body sizes were found during the experiment, the reproduction rate showed a significant difference in this case, as already shown in a previous study (Wang et al. 2022).

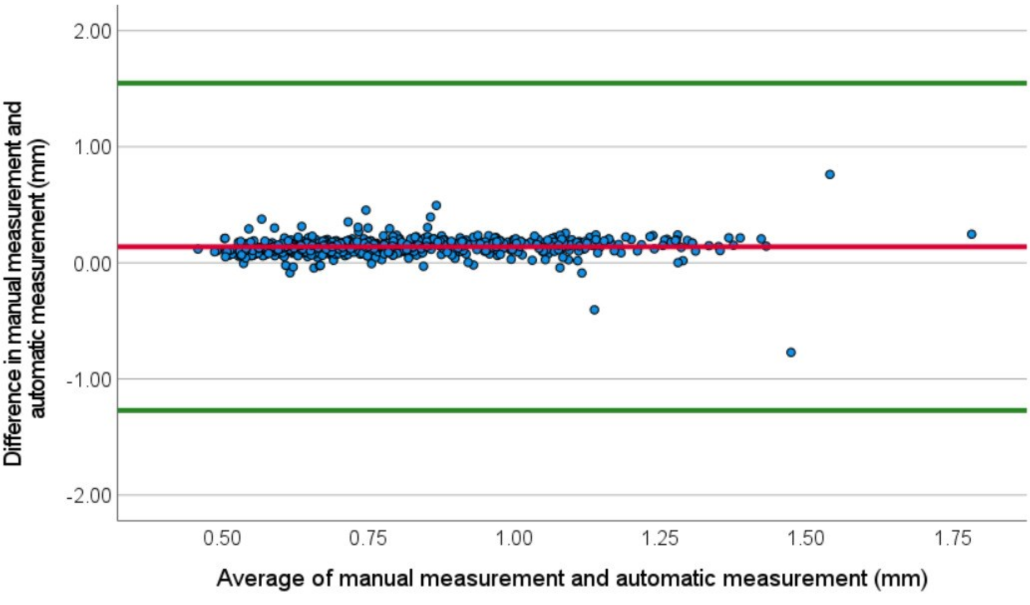


*Fig. 8* Bland-Altman plot for comparison of the automatic and manual measurements

The number of samples that can be processed can be greatly increased using the new device since the data can be processed immediately during extraction we can get. On the other hand, currently, sample processing after extraction can take weeks.

### *Measurement methods*

Our new digital extractor with Edapholog provides a novel approach that automatically measures microarthropods and estimates their body size. This device shows many novelties compared to previous automatic measurement methods. One of its advantages is that Edapholog registers 20 body-length data for each individual. Compared to another body size detecting device, e.g. CollScope, which saves only eight body length data (Bánszegi et al., 2014), the new device tested in our study provides a higher number of measurements and hence better reliable because more data is available to choose the right body size the animal has a greater chance of turning into the ideal body position.

Scientific studies related to automatic species identification and body size estimation have previously been published, showing that they can be well applied in practical ecology for measuring aquatic and terrestrial organisms in both field and laboratory conditions (Finlay et al. 2007, Agatz et al. 2015, Duckworth et al. 2019, Laursen et al. 2021). CollScope measured every individual in 30 seconds, and the measurement was semi-automatic as one person had to manage the device; the Edapholog device is automatic, and an individual's measurement lasts just for 2 seconds. Automatic measurements are usually performed on preserved individuals in a sample (Sys et al. 2022). The other advantage of Edapholog is that the animals can be used and extracted alive to be suitable for long-term ecotoxicological or drought tolerance studies and large sample numbers to work. This aspect makes Edapholog suitable for setting up long-term experiments with several generations of the test species. In addition, our new sample holder ("extracting sample holder") allows the animals to run out without disturbance, and the run-out time gives reliable results about the extracting process.

A minor systematic difference was found between the automatically and manually measured body length data. Fine-tuning the device can potentially overcome systematic differences in the future. However, the standard deviation of this difference was relatively large due to some outlier body size data. These outliers were mostly shorter but also longer than the normal body length values. The reason for these large deviations (> 0.3 mm) might be (1) that the AI could not adapt to recognize the unusual body positions of the collembolan individuals (Fig. 3/2.,3/4.). In these cases, the device largely underestimated the body sizes compared to the manual measurement. (2) In two cases, the animals fell into the cover of the blow-off opening, and the machine measured only part of their body (Fig. 3/3). Finally (3), the animals climbed up the funnel wall, so the device detected them as shorter than the normal body position (Fig. 3/1). In the future, these outliers might be corrected by a built-in filter that uses the body length-body width ratio of *F. candida*, and extreme deviations from normal ratios can be sorted out.

Finally, the outlier body lengths were sometimes higher than the mean values. Although the artificial intelligence follows the movement of the animals and can separate the falling *F. candida* individuals, in the case of two or more animals falling on top of each other, the AI program could not distinguish them and measured the two specimens as one. The simultaneous fall of several animals might be another reason for misdetection, which the further development of individual identification can solve.

This new Edapholog device allows us to follow the progress of soil-microarthropods' extraction by time stamps for every detection. There are many other possible experiments with the Edapholog, even with several species simultaneously, in which the dynamics of the extraction can also be tested. By further development, the use of Edapholog can greatly contribute to determining the species diversity and density of soil-dwelling invertebrates. Edapholog is a tool that can also be used in environmental ecology, which can greatly contribute to quick and precise data extraction.

## CONCLUSION

Although the applied drought treatments did not cause significant differences between the body sizes of treated *F. candida* groups, greater differences in drought levels might cause greater changes in body sizes and in species abundance, which might cause local extinction under natural conditions. Microarthropods play a significant role in soil carbon sequestration, so their decreasing presence in the soil may increase atmospheric carbon dioxide. The accuracy of the automatic body size estimation was high enough, making it suitable for automatic body size estimations. We measured only a small systematic error between the manual and automatic measurements, which can be corrected by further calibration.

## ACKNOWLEDGEMENT

The authors are grateful to Veronika Gergócs and Norbert Flórián for their help in the work. Thanks for Bernát Zawiasa for programming the automatic body size estimation and the Institute for Soil Sciences (Center for Agricultural Research) for providing the research environment. The experiment was financed by Hungarian Support grants for LIFE projects (project ID NYBZK/52/2023).

## REFERENCES

Agatz, A., Hammers-Wirtz, M., Gergs, A., Mayer, T., Preuss, T.G. 2015. Family-portraits for daphnids: Scanning living individuals and populations to measure body length. *Ecotoxicology* 24(6), 1385–1394. https://doi.org/10.1007/s10646-015-1490-0

Aszálymonitoring. (n.d.). Retrieved 6 July 2023, from https://aszalymonitoring.vizugy.hu/

Balla, E., Flórián, N., Gergócs, V., Gránicz, L., Tóth, F., Németh, T., Dombos, M. 2020. An Opto-Electronic Sensor-Ring to

Detect Arthropods of Significantly Different Body Sizes. *Sensors* 20(4), 982. https://doi.org/10.3390/s20040982

Bánszegi, O., Kosztolányi, A., Bakonyi, G., Szabó, B., Dombos, M. 2014. New Method for Automatic Body Length Measurement of the Collembolan, Folsomia candida Willem 1902 (Insecta: Collembola). *PLoS ONE* 9(6), e98230. https://doi.org/10.1371/journal.pone.0098230

Bardgett, R.D. 2005. The biology of soil: A community and ecosystem approach (34-37). Oxford University Press, New York (USA), p. 254.

Bayley, M., Petersen, S.O., Knigge, T., Köhler, H.-R., Holmstrup, M. 2001. Drought acclimation confers cold tolerance in the soil collembolan Folsomia candida. *Journal of Insect Physiology* 47(10), 1197–1204. https://doi.org/10.1016/S0022-1910(01)00104-4

Bilyk, Z.I., Shapovalov, Y.B., Shapovalov, V. B., Megalinska, A.P., Andruszkiewicz, F., Dołhańczuk-Śródka, A. 2020. Assessment of mobile phone applications feasibility on plant recognition: Comparison with Google Lens AR-app. [б. в.]. *CEUR Workshop Proceedings* 2020. Vol. 2731, 61–78. https://doi.org/10.31812/123456789/4403

Briones, M. J.I. 2014. Soil fauna and soil functions: A jigsaw puzzle. *Frontiers in Environmental Science* 2. https://doi.org/10.3389/fenvs.2014.00007

Brussaard, L., de Ruiter, P.C., Brown, G.G. 2007. Soil biodiversity for agricultural sustainability. *Agriculture, Ecosystems & Environment* 121(3), 233–244. https://doi.org/10.1016/j.agee.2006.12.013

Christin, S., Hervet, É., Lecomte, N. 2019. Applications for deep learning in ecology. *Methods in Ecology and Evolution* 10(10), 1632–1644. https://doi.org/10.1111/2041-210X.13256

Coleman, D.C., Callaham, M.A., Jr, Crossley, D.A., Jr. 2017. Fundamentals of Soil Ecology. Academic Press. https://doi.org/10.1016/C2015-0-04083-7

Crossley, D.A., Blair, J.M. 1991. A high-efficiency, "low-technology" Tullgren-type extractor for soil microarthropods. *Agriculture, Ecosystems & Environment* 34(1–4), 187–192. https://doi.org/10.1016/0167-8809(91)90104-6

Dombos, M., Kosztolányi, A., Szlávecz, K., Gedeon, C., Flórián, N., Groó, Z., Dudás, P., Bánszegi, O. 2017. EDAPHOLOG monitoring system: Automatic, real-time detection of soil microarthropods. *Methods in Ecology and Evolution* 8(3), 313–321. https://doi.org/10.1111/2041-210X.12662

Duckworth, J., Jager, T., Ashauer, R. 2019. Automated, high-throughput measurement of size and growth curves of small organisms in well plates. *Scientific Reports* 9(1), 10. https://doi.org/10.1038/s41598-018-36877-0

Filser, J. 2002. The role of Collembola in carbon and nitrogen cycling in soil. *Pedobiologia* 46(3–4), 234–245. https://doi.org/10.1078/0031-4056-00130

Finlay, K., Beisner, B.E., Barnett, A.J.D. 2007. The use of the Laser Optical Plankton Counter to measure zooplankton size, abundance, and biomass in small freshwater lakes: LOPC in freshwater lakes. *Limnology and Oceanography: Methods* 5(1), 41–49. https://doi.org/10.4319/lom.2007.5.41

Flórián, N., Gránicz, L., Gergócs, V., Tóth, F., Dombos, M. 2020. Detecting Soil Microarthropods with a Camera-Supported Trap. *Insects* 11(4), 244. https://doi.org/10.3390/insects11040244

Gedeon, C., Flórián, N., Liszli, P., Hambek-Oláh, B., Bánszegi, O., Schellenberger, J., Dombos, M. 2017. An Opto-Electronic Sensor for Detecting Soil Microarthropods and Estimating Their Size in Field Conditions. *Sensors* 17(8), 1757. https://doi.org/10.3390/s17081757

Hilligsøe, H., Holmstrup, M. 2003. Effects of starvation and body mass on drought tolerance in the soil collembolan Folsomia candida. *Journal of Insect Physiology* 49(1), 99–104. https://doi.org/10.1016/S0022-1910(02)00253-6

Høye, T.., Ärje, J., Bjerge, K., Hansen, O.L.P., Iosifidis, A., Leese, F., Mann, H.M.R., Meissner, K., Melvad, C., Raitoharju, J. 2021. Deep learning and computer vision will transform entomology. *Proceedings of the National Academy of Sciences* 118(2), e2002545117. https://doi.org/10.1073/pnas.2002545117

IBPM SPSS Statistics for Windows (27.0). (2020). IBM Corp.

ISO (1999) Soil quality–Inhibition of reproduction of Collembola (Folsomia candida) by soil pollutants. ISO 11267. Geneva: International Standardization Organization.

ISO (2006) Soil quality–Sampling of soil invertebrates – Part 2: Sampling and extraction of micro-arthropods (Collembola and Acarina). ISO 23611-2: 2006. Geneva: International Standardization Organization.

ISO (2011) Soil quality–Avoidance test for determining the quality of soils and effects of chemicals on behaviour–Part 2: Test with collembolans (Folsomia candida). ISO 17512-2: 2011. Geneva: International Standardization Organization.

Kardol, P., Reynolds, W.N., Norby, R.J., & Classen, A.T. 2011. Climate change effects on soil microarthropod abundance and community structure. *Applied Soil Ecology* 47(1), 37–44. https://doi.org/10.1016/j.apsoil.2010.11.001

Laursen, S.F., Hansen, L.S., Bahrndorff, S., Nielsen, H. M., Noer, N.K., Renault, D., Sahana, G., Sørensen, J.G., Kristensen, T.N. 2021. Contrasting Manual and Automated Assessment of Thermal Stress Responses and Larval Body Size in Black Soldier Flies and Houseflies. *Insects* 12(5), 380. https://doi.org/10.3390/insects12050380

Liu, Z., Peng, C., Work, T., Candau, J.-N., DesRochers, A., Kneeshaw, D. 2018. Application of machine-learning methods in forest ecology: Recent progress and future challenges. *Environmental Reviews* 26(4), 339–350. https://doi.org/10.1139/er-2018-0034

Lock, K., Janssen, C.R. 2002. Ecotoxicity of nickel to Eisenia fetida, Enchytraeus albidus and Folsomia candida. *Chemosphere* 46(2), 197–200. https://doi.org/10.1016/S0045-6535(01)00112-6

Makkonen, M., Berg, M.P., van Hal, J.R., Callaghan, T.V., Press, M.C., Aerts, R. 2011. Traits explain the responses of a sub-arctic Collembola community to climate manipulation. *Soil Biology and Biochemistry* 43(2), 377–384. https://doi.org/10.1016/j.soilbio.2010.11.004

Mallard, F., Le Bourlot, V., Tully, T. 2013. An Automated Image Analysis System to Measure and Count Organisms in Laboratory Microcosms. *PLoS ONE* 8(5), e64387. https://doi.org/10.1371/journal.pone.0064387

Meehan, M.L., Barreto, C., Turnbull, M.S., Bradley, R. L., Bellenger, J.-P., Darnajoux, R., Lindo, Z. 2020. Response of soil fauna to simulated global change factors depends on ambient climate conditions. *Pedobiologia* 83, 150672. https://doi.org/10.1016/j.pedobi.2020.150672

Menta, C., Conti, F.D., Pinto, S., Bodini, A. 2018. Soil Biological Quality index (QBS-ar): 15 years of application at global scale. *Ecological Indicators* 85, 773–780. https://doi.org/10.1016/j.ecolind.2017.11.030

Ninon, R., Cyrille, D., Phillip, B. 2019. Fossil amber reveals springtails' longstanding dispersal by social insects [Preprint]. *Paleontology*. https://doi.org/10.1101/699611

OECD, 2009. OECD Guidelines for Testing Chemicals 232 Collembolan Reproduction Test in Soil.

Pereira, P., Bogunovic, I., Muñoz-Rojas, M., Brevik, E.C. 2018. Soil ecosystem services, sustainability, valuation and management. *Current Opinion in Environmental Science & Health* 5, 7–13. https://doi.org/10.1016/j.coesh.2017.12.003

Silvertown, J. 2009. A new dawn for citizen science. *Trends in Ecology & Evolution* 24(9), 467–471. https://doi.org/10.1016/j.tree.2009.03.017

Sjursen, H., Bayley, M., Holmstrup, M. 2001. Enhanced drought tolerance of a soil-dwelling springtail by pre-acclimation to a mild drought stress. *Journal of Insect Physiology* 47(9), 1021–1027. https://doi.org/10.1016/S0022-1910(01)00078-6

Sørensen, T.S., Holmstrup, M. 2005. A comparative analysis of the toxicity of eight common soil contaminants and their effects on drought tolerance in the collembolan Folsomia candida. *Ecotoxicology and Environmental Safety* 60(2), 132–139. https://doi.org/10.1016/j.ecoenv.2004.02.001

Steffen, W., Crutzen, P.J., McNeill, J.R. 2007. The Anthropocene: Are Humans Now Overwhelming the Great Forces of Nature? *Ambio* 36(8), 614–621. https://doi.org/10.1579/0044-7447(2007)36[614:TAAHNO]2.0.CO;2

Sys, S., Weißbach, S., Jakob, L., Gerber, S., Schneider, C. 2022. CollembolAI, a macrophotography and computer vision workflow to digitize and characterize samples of soil invertebrate communities preserved in fluid. *Methods in*

*Ecology and Evolution* 13(12), 2729–2742. https://doi.org/10.1111/2041-210X.14001

Szabó, B., Bálint, B., Balogh, K., Mézes, M., Seres, A. 2022. Changes in soil moisture and temperature modify the toxicity of sodium selenite and sodium selenate for Folsomia candida (Collembola) Willem 1902. *Applied Soil Ecology* 177, 104543. https://doi.org/10.1016/j.apsoil.2022.104543

Wang, Y., Slotsbo, S., Holmstrup, M. 2022. Soil dwelling springtails are resilient to extreme drought in soil, but their reproduction is highly sensitive to small decreases in soil water potential. *Geoderma* 421, 115913. https://doi.org/10.1016/j.geoderma.2022.115913