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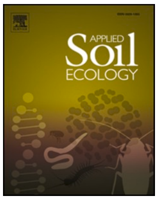

What is the best way to measure earthworm-processed soil? A comparison of common water stable aggregates, the smartphone app MOULDER, and a novel SlakeLight method

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ABSTRACT

Soil aggregates are important for soil fertility and earthworms can support aggregate formation and stability during gut passage, burrowing activity and secretion of polysaccharides. To determine these effects in different soil tillage systems, soil properties, and the earthworm community associated with *Lumbricus terrestris* (Linnaeus, 1758) were evaluated in two long-term field experiments using plough, cultivator, or no-till. The aim was to investigate effects of earthworm populations on soil aggregate stability and to evaluate three methods, namely: water stable aggregate (WSA) index, MOULDER (formerly SLAKES) and SlakeLight. The WSA method wet-sieves aggregates for 3 min in distilled water and records mass of the dispersed aggregates. MOULDER uses a smartphone application to measure an increase in area of dispersed soil submerged in distilled water to measure slaking, while SlakeLight analyses light transmission corresponding to the area covered by the soil material. Soil samples were collected in autumn at three levels: i) middens, ii) 5 cm radius around burrows of *L. terrestris* (burrow-midden-complex), and iii) in bulk soil without burrows of *L. terrestris*. All samples were hand-searched for earthworms or air-dried for soil aggregate stability analyses. The hypothesis tested was that there is a gradient of earthworm worked soil, such that the level of earthworm activity away from a *L. terrestris* burrow decreases so that: midden > burrow-midden-complex > bulk soil. Total earthworm abundance (individuals level^{-1} ± standard deviation), mostly endogeic earthworms, was 3-times higher in the burrow-midden-complex (4.3 ± 2.7) than bulk soil for cultivator and doubled for burrow-midden-complex under a ploughing regime (3 ± 2.1), while no-till was only slightly increased (4.6 ± 2). With rising earthworm numbers, aggregate stability increased, with a higher effect for bulk soil than for burrow-midden-complex. At both sites, MOULDER identified a more stable soil in middens than in bulk soil, while other methods were not so discriminating in their outcomes of middens. However, WSA was more sensitive to interactions of soil tillage × earthworm abundance than SlakeLight or MOULDER and showed that ploughed soil and bulk soil aggregates stabilised the most with increasing earthworm activity. Comparison of the three methods showed that all can be used for earthworm-processed soil, but that selection of the method should depend on the research questions and on resource availability.

1. Introduction

Soil aggregates contribute to soil fertility (Amézketa, 1999; Bronick and Lal, 2005) and earthworms are known to increase soil aggregate stability (Arai et al., 2018). Methods to measure soil aggregates are manifold and comparisons between some methods such as Cornell Rainfall Simulator (Moebius-Clune et al., 2016), wet sieve procedure (Nimmo and Perkins, 2002) and MOULDER (formerly SLAKES; Version 2.0) (Fajardo et al., 2016; Fajardo and McBratney, 2023) have been made, but mostly from different soil tillage systems and farming practices (e.g., Flynn et al., 2020; Rieke et al., 2022) and not for earthworm-processed soil. Previous comparisons between these methods showed that results varied between the investigated management systems (Flynn et al., 2020) and Rieke et al. (2022) stressed that determination of the

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different method sensitivities, due to origin of soil samples, are key to understand their role as indicators of soil fertility.

It is well known that soil aggregate stability and earthworm abundance decrease with soil tillage intensification (Briones and Schmidt, 2017; Sae-Tun et al., 2022) and that earthworms can form and stabilise soil aggregates through burrowing and casting (Schrader and Zhang, 1997; Six et al., 2004; Lehmann et al., 2017). These effects can vary between the earthworm ecological groups (viz.: anecic; endogeic; epigeic; Bottinelli and Capowiez, 2021) according to their given characteristics in burrowing and feeding behaviour (Bouché, 1972, 1977). Epigeic earthworms are litter dwellers and therefore have relatively little direct effect on aggregate stability compared to other groups (Shipitalo and Le Bayon, 2004), while *Lumbricus terrestris* (Linnaeus, 1758), a prominent example of the anecic group, can increase soil aggregates (Ketterings et al., 1997). *L. terrestris* has a permanent vertical burrow and is a fresh litter feeder (Capowiez et al., 2003, 2011; Butt and Nuutinen, 2005), it builds middens on the soil surface primarily consisting of casts and plant residues (Butt and Nuutinen, 2005; Nuutinen and Butt, 2019). These middens and burrows (the burrow-midden-complex; Butt and Nuutinen, 2005) are hotspots for endogeic earthworms (Butt and Lowe, 2007) and microorganisms, both related to nutrient availability and polysaccharide content of the mucilage of earthworms, which leads to increased amounts of dissolved organic carbon (Bohlen et al., 1997; Vos et al., 2014; Stroud et al., 2016). Dissolved organic carbon and polysaccharides stabilise soil aggregates through turnover of microorganism biomass to necromass (Sae-Tun et al., 2022). However, endogeic earthworms, the third ecological group, have more horizontal, temporary burrows in the mineral soil layer, which are partly filled with casting (Capowiez et al., 2014). In a pot experiment of Hallam and Hodson (2020), endogeic earthworms viz. *Allolobophora chlorotica* (Savigny, 1826) showed a similar increase in aggregate stabilisation to *L. terrestris*. However, under field conditions (effects of) ecological groups cannot be separated, as Butt and Lowe (2007) found a higher amount of different earthworm species under middens and around burrows of *L. terrestris* (burrow-midden-complex) than in bulk soil without middens. These findings indicate that the *L. terrestris* burrow-midden-complex is of interest for anecic, endogeic and epigeic earthworms and forms the justification for the current study. It was hypothesised that soil has a higher soil stability in midden > burrow-midden-complex > bulk soil related to the activity level of earthworms, and this is independent of soil tillage systems.

The current study compared the sensitivity of i) common water stable aggregates (Nimmo and Perkins, 2002), ii) soil slaking measured by the smartphone application MOULDER (Fajardo et al., 2016; Fajardo and McBratney, 2023) and iii) SlakeLight (Madaras et al., 2024) to earthworm-processed soil at three levels (middens; burrow-midden-complex; bulk soil), within three soil tillage systems (cultivator; no-till; plough). The aim of this study was to identify a method to test relationships of soil stability and earthworm abundance in and around *L. terrestris* middens under different soil tillage regimes, as soil aggregates are vitally important in fully functioning soils (Amézketa, 1999; Bodner et al., 2023).

2. Materials and methods

2.1. Experimental set-up, sites and soil sampling

The soil samples for comparison of the aggregate stability methods originated from two long-term tillage experiments in Hollabrunn (site 1; 48°34′N, 16°5′E; instigated in 2006) and in Raasdorf (site 2; 48°14′N, 16°33′E; instigated in 1996), in north-east Austria. Both sites are in the Pannonian climate zone with mean annual temperature of 10.15 and 11.21 °C and a mean annual precipitation of 517 and 560 mm (site 1 and 2, respectively) (GeoSphere Austria, 2023) and a chernozem soil of loamy silt texture (WRB, 2014) with clay 217; 220 g kg^{-1}, silt 570; 582 g kg^{-1}, sand 198; 213 g kg^{-1}, pH_{CaCl2}: 7.5; 7.6, total organic carbon 23.5; 26.5 g kg^{-1} of site 1 and 2, respectively (Yu et al., 2016; Rosner et al., 2018). At both sites, there was a randomised block design, with 3 and 4 blocks (site 1 and 2, respectively) and three soil tillage treatments of plough (30 cm depth), cultivator (10 cm depth) and no-till (0 cm depth). Plot sizes were 6 × 50 m and 20 × 40 m at site 1 and site 2, respectively.

2.2. Soil and earthworm sampling

At the time of sampling in November 2019 or 2020 at both sites, maize (*Zea mays* L.) had just been harvested. Soil samples were taken from i) bulk soil without middens, ii) middens and iii) the burrow-midden-complex at a 5 cm radius from the burrow, below the midden by use of a heavy-duty hammer and a soil auger of 10 cm diameter and 10 cm depth. Soil samples were then air-dried for 2 months and sieved to 2.5–5 mm. Due to the lack of plant residues in plough and cultivator plots, wheat straw (178 g m^2) was added to all plots to an area of 7 m^2 in May and in June 2019 or 2020 to enable definitive identification of middens of *L. terrestris*. (These were easily located, as the resident earthworms collected this straw to their middens.)

Earthworms were only sampled at site 2, using the same method (heavy-duty hammer and soil auger) as described for soil sampling. Five subsamples were taken per midden, burrow-midden-complex and bulk soil per plot. First, middens were sampled from the soil surface, before burrow-midden-complex samples from under the middens were taken. The bulk soil originated from an area without a midden from the same plot, which proved to be difficult in no-till plots with an average density of 30 middens m^{-2}. Earthworms were hand-searched for in midden, burrow-midden-complex and bulk soil, then washed and carefully blotted dry. Earthworms were counted and biomass was recorded of juveniles and adults, while adults were also identified to species level using keys of Christian and Zicsi (1999) and Sherlock (2018).

2.3. Method: water stable aggregates

Aggregate stability was assessed using the water-stable aggregates index (WSA) proposed by Nimmo and Perkins (2002). Prior to measurement air-dried soil samples were sieved to extract aggregates with diameters of 2–5 mm. Four grams of sieved aggregates (4 replicates) were placed on the sieves (height 3.9 cm, diameter 3.9 cm) of Wet Sieving Apparatus (sieve 0.25 mm) and washed in cans (stainless steel; height 4.5 mm; diameter 3.2 cm and volume 144.8 cm^3) with distilled water for 3 min. These cans were then replaced with cans with a dispersing solution (containing 2 g sodium hexametaphosphate l^{-1} for soils with pH > 7 and 2 g sodium hydroxide l^{-1} for soils with pH < 7) and the sieving continued until only the individual mineral particles (and root fragments) were left on the sieves. Particles larger than 0.25 mm such as sand are excluded from the index, due to this procedure. Both sets of cans were placed in an oven and dried at 110 °C. After drying, the weight of materials of unstable and stable aggregates (without particles >0.25 mm) was determined. Dividing the weight of the stable aggregates over the total aggregate weight after sieving gives an index for the aggregate stability (Eq. 1):

$$WSA = \frac{W_{ds}}{W_{ds} + W_{dw}} \tag{1}$$

where W_{ds} is the weight of aggregates dispersed in dispersing solution and W_{dw} is the weight of aggregates dispersed in distilled water. A WSA value of 1 represent the most stable soil aggregate.

2.4. Method: MOULDER

Soil was analysed using the smartphone application MOULDER (Fajardo et al., 2016; Fajardo and McBratney, 2023) following the analytical protocol of Flynn et al. (2020) and using an iPhone 7 (Apple

Inc., Cupertino, CA, USA). In brief, for the measurement, air-dried soil was sieved (2–5 mm) and three soil aggregates per sample and five subsamples per plot were used. The first step of the protocol is a reference orthophoto of the dry soil aggregates in a dry Petri dish in front of a high contrast background (i.e., a white sheet of paper). In a second step, aggregates were transferred into a Petri dish filled with deionised water. While transferring the aggregates, they should be placed in the same position and direction as in the reference image. The application measures the increasing area of the dispersed soil with the reference image as start and the final area after 10 min. It is therefore important to avoid shadows, as these can be misinterpreted as soil. This was achieved with two desk lamps and the smartphone was placed above the Petri dish to comprise the entire viewing frame of the phone. After ten minutes the α-coefficient is shown on the display. The α-coefficient is the maximum predicted dispersion of a soil aggregate and derived from the Slaking index fitted to the Gompertz function (Gompertz, 1825). For details of the α-coefficient, the Slaking index and the method, see Fajardo et al. (2016). A low α-coefficient represents more stable soil with zero as the most stable outcome.

2.5. Method: SlakeLight

A SlakeLight method for determination of soil aggregate stability was developed and tested at the Crop Research Institute in Prague. The principle of the patented invention SlakeLight (Madaras and Krejčí, 2020) is detailed by Madaras et al. (2024). SlakeLight works on the principle of measuring light transmittance within a water-filled measuring chamber, in which nine soil aggregates of 3–4 mm size slowly dissolve. Dissolving soil aggregate shades the light during its gradual slaking. The light source used needs to have a surface-homogeneous distribution of luminosity. The process of disintegration of the soil aggregates results in a reduction of light transmission through the measuring vessel to a degree exactly corresponding to the area covered by the soil material. Determination of the aggregate slaking rate is possible by measuring the light transmission through the measuring vessel, i.e., by measuring the decrease in light flux above the vessel by photodiode. Light transmittance was measured after 2, 4 and 10 min and voltages were recorded at the beginning (U_1) and end (U_2) of each measurement (Eq. 2). The result is given as SAS_{trans}

$$SAS_{trans} = \frac{100^* U_2}{U_1} \tag{2}$$

the median from nine soil aggregates and corresponds to the stability of the soil aggregates. The highest value of 100 is displayed when no disintegration of aggregates occurs. The higher the value is, the more stable the soil aggregates are.

2.6. Data analyses

To provide an overview about the structure of the data, the variance of coefficients of soil aggregate stability of methods were compared in a three-way linear mixed model (3-way LMM) with fixed factor method (MOULDER; WSA; SlakeLight), earthworm-processed soil levels (bulk soil; burrow-midden-complex; midden) and soil tillage (plough; cultivator; no-till) and random factor site (site 1; site 2) (Piepho et al., 2003). In addition, the relationship between the methods were tested by Pearson correlation and pairwise scatterplots to show the relation of the data gained from the methods (function ‘ggpairs’, package ‘*GGally*’; Schloerke et al., 2021) (Piepho, 2018; Rieke et al., 2022).

The data of soil aggregate stability for MOULDER and WSA were analysed with 3-way LMM with fixed factors site, processed level and soil tillage and block as random factor. For SlakeLight time factor (3 levels; 2 min; 4 min; 10 min) were used in random factors (Piepho et al., 2004). To analyse earthworm parameters of site 2, a two-way LMM (2-way LMM) was applied with fixed factors; sampling level (bulk soil; burrow-midden-complex; midden), soil tillage and block as random factor. Linear MMs considered site, block and plot or block and plot in compound symmetry for the variance covariance structure (Piepho et al., 2003, 2004). Normal distribution of residual was inspected in QQ-plots and homogeneity of the variance was determined by plotting residuals against fitted values. WSA and LED met the assumptions and only MOULDER was square root transformed. Linear MM was performed with ‘lmer’ (‘*lme4*’ package) (Bates et al., 2015) in RStudio 6.1.524 (Posit team, 2023) using R 4.3.1 (R Development Core Team, 2024) using the residual maximum likelihood method. Function ‘anova’ (with type III hypotheses) was used for ANOVA with Wald-type *F*-tests and Satterthwaite's method to obtain denominator degrees of freedom. For the Tukey test for pairwise comparisons of factors ($P < 0.05$), function ‘emmeans’ (package ‘*emmeans*’) (Lenth, 2022) and ‘cld’ (package ‘*multcomp*’) (Hothorn et al., 2008) was used.

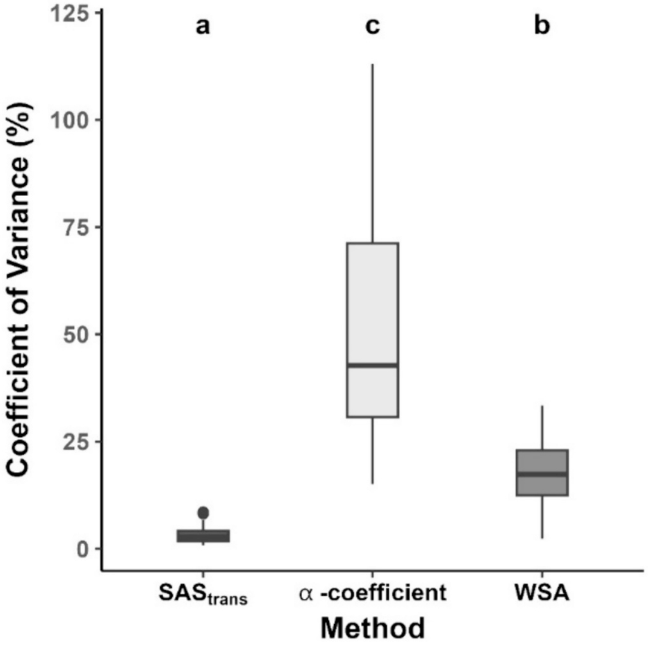

Fig. 1. Boxplot of coeffiecient of variance from a method comparison of α-coeffiecient, water stable aggregates (WSA) and SlakeLight (SAS_{trans}) across earthworm processed soil levels (bulk soil; burrow-midden-complex; midden) and soil tillage system (cultivator; no-till; plough) at two sites in Austria. Methods having no letter in common are significantly different by pairwise comparison (three-way linear mixed model, Tukey; $P < 0.05$; $N = 7$).

To model the soil aggregate stability as a function of total earthworm abundance, a 3-way LMM regression with fixed factors: soil tillage, processed level (bulk soil vs. burrow) and earthworm abundance, and random effects replicate and plot (Piepho, 2018). In a three-step process, the full model was reduced by removing the 3-way and then the 2-way interaction term when not significant (function ‘anova’ with type I hypotheses and Kenward–Roger's method) (Euteneuer et al., 2022). Middens were excluded from regression, because middens mostly consist of cast of *L. terrestris* and do not host great numbers of any earthworms and could therefore lead to dubious conclusions. In the last step of the regression, all interaction terms were removed and only soil tillage, processed level or earthworm abundance was tested. Protocols of Zuur et al. (2010) and Zuur and Ieno (2016) were followed to check for assumptions. Figures were drawn with ‘plot_model’ type ‘emm’ of the packages ‘*sjPlot*’ (Lüdecke, n.d.) and ‘ggplot2’ (Wickham, 2016).

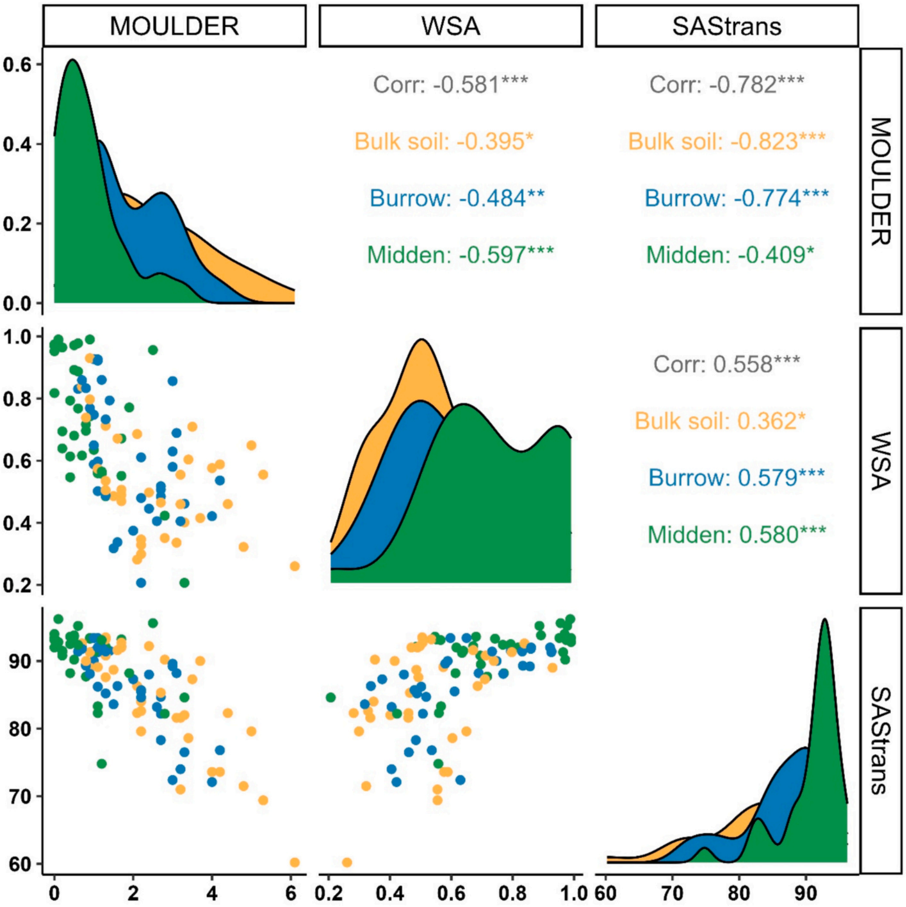

Fig. 2. Matrix with density plot and Pearson correlation with given *r* of α-coeffiecient (MOULDER), water stable aggregates (WSA) and SlakeLight (SAS_{trans}) across soil tillage systems (cultivator; no-till; plough) at two sites in Austria for earthworm-processed soil levels (bulk soil; burrow-midden-complex (burrow); midden) or overall correlation (Corr). *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

Table 1
Results of three-way linear mixed model of soil aggregate stability of method MOULDER (α-coefficient), water stable aggregate method (WSA) or SlakeLight (SAS_{trans}) with fixed factors soil tillage systems (T: cultivator; no-till; plough), site (S: site 1; site 2) and earthworm-processed soil level (L: bulk soil; burrow-midden-complex; midden). Degrees of freedom for both treatment and error terms (Df), *F*-values and *P*-values., $N_{site1} = 3$ and $N_{site2} = 4$.

Parameter	Treatment	Df	*F*-value	*P*-value
α-coefficient	Tillage (T)	2.18	16.1	< 0.001
	Level (L)	2.20	50.1	< 0.001
	Site (S)	1.18	13.9	< 0.001
	T × L	4.20	4.05	0.003
	T × S	2.18	0.34	0.716
	L × S	2.20	12.9	< 0.001
	T × L × S	4.20	5.40	< 0.001
WSA	T	2.18	7.30	0.005
	L	2.20	4.95	0.008
	S	1.18	0.091	0.767
	T × L	4.20	4.26	0.002
	T × S	2.18	0.606	0.556
	L × S	2.20	5.07	0.007
	T × L × S	4.20	2.82	0.026
SAS_{trans}	T	2.32	16.1	< 0.001
	L	2.86	50.1	< 0.001
	S	1.32	13.9	< 0.001
	T × L	4.86	4.05	0.003
	T × S	2.32	0.337	0.716
	L × S	2.86	12.9	< 0.001
	T × L × S	4.86	5.40	< 0.001

3. Results

3.1. Comparison of MOULDER, WSA and SlakeLight

Methods alone affected coeffiecient of variance, with the highest coeffiecient of variance for α-coeffiecient followed by WSA > SAS_{trans} ($F_2 = 12.1$, $P < 0.001$; Fig. 1). WSA and SlakeLight had an inverse relationship with α-coeffiecient in all processed levels from $r = -0.395$ to - 0.823 (Fig. 2). Overall, α-coeffiecient had a moderately negative correlation with WSA and was strongly negatively correlated with SlakeLight, while WSA and SlakeLight were moderately positively correlated (Fig. 2).

3.2. Earthworm stabilised soil aggregates and earthworm abundance

Overall, soil aggregate stability was affected by interactions of site, soil tillage and level for all methods (Table 1) and found similar trends for earthworm-processed soil levels with more stable soil aggregates in midden followed by burrow-midden-complex and then bulk soil. In detail, for α-coeffiecient (Fig. 3 A) all middens were 3-times more stable than bulk soil, while WSA and SAS_{trans} were 1.4–1.5-times more stable at both sites for plough (Fig. 3 B,C). In addition, contrasts between burrow-midden-complex and bulk soil were less distinct for α-coeffiecient (except cultivator at site 2), SAS_{trans} (except cultivator at site 1) and for WSA (except cultivator and no-till at site 2). Middens were 1.45–2-times more stable than burrow-midden-complex at site 2, except for α-coeffiecient in cultivator and plough and were similar at site 1 except for SlakeLight in cultivator (Fig. 3).

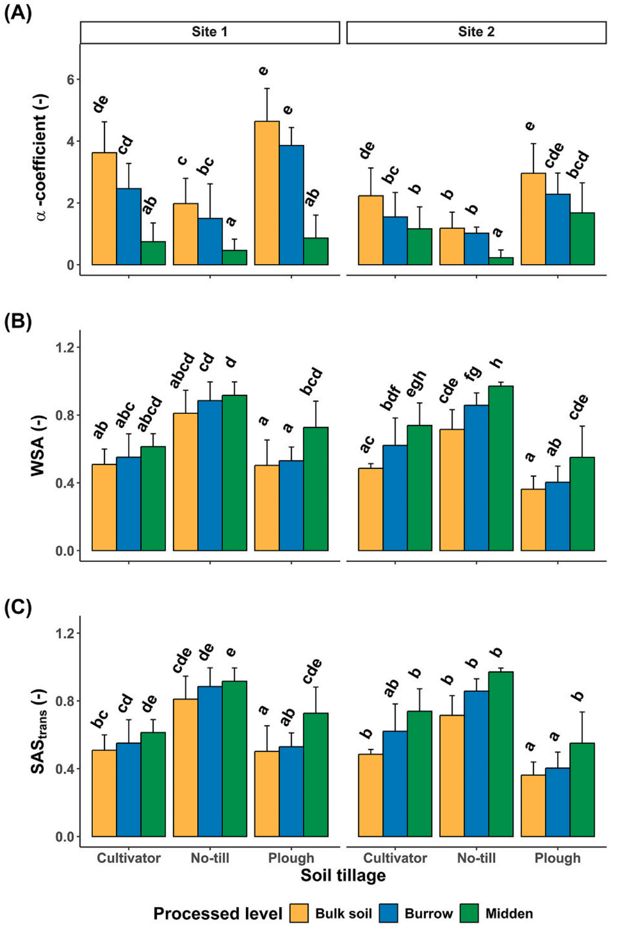

Fig. 3. Soil aggregate stabilisation of earthworm-processed soil (bulk soil; burrow-midden-complex (burrow); midden) analysed by method MOULDER (A; α-coefficient), water stable aggregate (B; WSA) or SlakeLight (C; SAS$_{\text{trans}}$). Note: α-coefficient has an inverse relationship with WSA and SAS$_{\text{trans}}$. Processed levels per site having no letter in common are significantly different by pairwise comparison (three-way linear mixed model, Tukey; $P < 0.05$). Values are mean + SD ($N_{\text{site1}} = 3$; $N_{\text{site2}} = 4$).

Table 2
Proportion of the earthworm community found with hand-searching in middens ($n = 38$ individuals level^{-1}) and the burrow-midden-complex ($n = 222$ individuals level^{-1}) of *Lumbricus terrestris* or bulk soil ($n = 113$ individuals level^{-1}) in November 2020 at site 2.

Earthworm community	Midden (%)	Burrow (%)	Bulk soil (%)	Overall mean (%)
Endogeic, juvenile	81	84.5	87	84.2
Endogeic, adult	16	11	11	12.7
Anecic, juvenile	3	4	2	3.0
L. terrestris, adult	0	0.5	0	0.2

Table 3
Results of two-way linear mixed model of total or endogeic earthworm abundance or biomass with fixed factors soil tillage systems (T: cultivator; no-till; plough) and earthworm-processed soil level (L: bulk soil; burrow-midden-complex; midden) of site 2. *F*-values and *P*-values, $N = 4$. Degrees of freedom for both treatment and error terms (Df).

Parameter	Treatment	Df	*F*-value	*P*-value
Total abundance	Soil tillage (T)	2.41	5.75	0.006
	Level (L)	2.16	26.8	< 0.001
	T × L	4.16	1.96	0.103
Total biomass	T	2.31	4.20	0.024
	L	2.159	17.2	< 0.001
	T × L	4.16	2.37	0.054
Endogeic abundance	T	2.40	5.43	0.008
	L	216	25.7	< 0.001
	T × L	4.16	2.02	0.094
Endogeic biomass	T	2.30	4.76	0.016
	L	2.16	16.0	< 0.001
	T × L	4.16	3.11	0.017

The earthworm community at site 2 for the middens, burrow-midden-complex and bulk soil comprised of endogeic species (97 %) such as *Aporrectodea caliginosa* (Savigny, 1826), *A. rosea* (Savigny, 1826) and *Allolobophora chlorotica* and 3 % anecic earthworms, with a higher number of juveniles (87 %) than adults (13 %) (Table 2). Total earthworm abundance, biomass and endogeic earthworm abundance at site 2 showed strong effects of soil tillage, earthworm-processed soil level, but no interactions, except for endogeic biomass (Table 3). In detail, highest total earthworm abundance was found around the burrow-midden-complex of *L. terrestris* in each of the soil tillage systems (Fig. 4 A). A similar situation was observed for endogeic earthworm abundance, but only for cultivator and plough (Fig. 4 C). Overall, bulk soil and middens had a similar number for total and endogeic abundance, except for no-till, where abundance in bulk soil was 4.5 -times higher than in middens (Fig. 4 A,C). In addition, total biomass and endogeic biomass followed a similar pattern to abundance, but showed no differences for bulk soil and burrow, in no-till and plough (Fig. 4 B,D).

Regression models showed that total earthworm abundance affected soil aggregate stability in all methods differently (Table 4). Soil aggregates stability for α-coefficient, WSA and SAS$_{\text{trans}}$ were affected by processed soil levels, while α-coefficient was also affected by soil tillage (Fig. 5 B) and processed soil level × tillage for WSA (Fig. 5 D). For α-coefficient and SAS$_{\text{trans}}$ the increase of aggregate stability only applied for bulk soil, but not for the burrow-midden-complex (Fig. 5 A,C). A decrease was seen in the burrow-midden-complex for WSA at no-till and cultivator (Fig. 5 D; Supplementary Table S1). In addition, α-coefficient for plough decreased and aggregates became more stable in the presence of earthworms compared to no-till (Fig. 5 B; Supplementary Table S1). Overall, similar results were observed for endogeic earthworms (Data not shown).

4. Discussion

4.1. Method comparison

The comparison of three methods for soil aggregate stability showed that all were sensitive to soil tillage, sites and earthworm-processed soil to different degrees. Water stable aggregates and MOULDER found 12 significant differences out of 18 variants and SlakeLight resulted in 13 significant differences. These results were similar for all methods and were underlined by moderate to strong correlations between the methods. Rieke et al. (2022) reported similar outcomes for a comparison of different soil tillage system between Cornell Rainfall Simulator (Moebius-Clune et al., 2016), wet sieve procedure (Kemper and Rosenau, 1986), water stable aggregate mean weight diameter (Franzluebbers et al., 2000) and MOULDER. Rieke et al. (2022) concluded that no single method was superior and that secondary aspects such as resource (human and monetary) availability and within-treatment variability are determinate for method selection. MOULDER, in the current study, had the highest coefficient of variance, but this was mostly affected by the more distinct differences between bulk soil and middens compared to WSA or SlakeLight. Middens for MOULDER were 2.94-times more stable than bulk soil, while middens analysed with WSA and SlakeLight were only 1.38-times more stable than bulk soil.

Results of methods can be affected by climate and soil properties (Rieke et al., 2022) and annual precipitation at site 1 was 25 % lower than at site 2, while soil type, texture and pH-value were similar at the sites. In addition, samples at both sites were taken after the maize

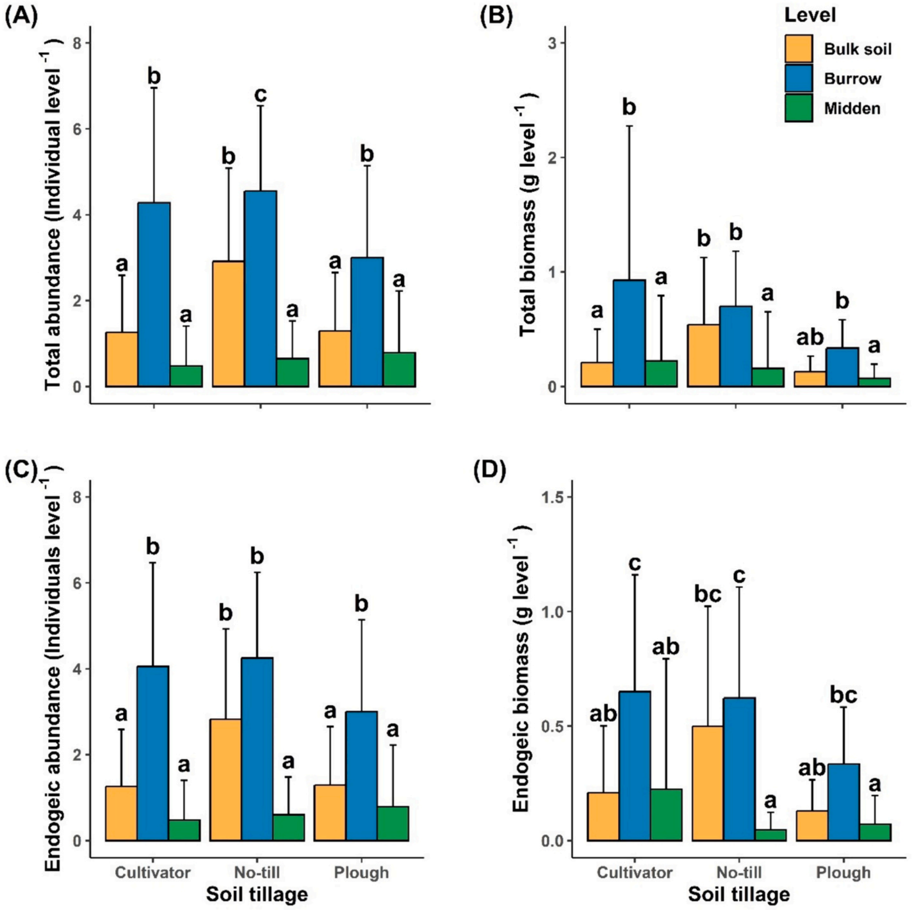

Fig. 4. Total earthworm abundance (A), total biomass (B), endogeic abundance (C) and endogeic biomass (D) per sampling level (bulk soil; burrow-midden-complex (burrow); midden) of site 2. Levels per soil tillage having no letter in common are significantly different by pairwise comparison (two-way linear mixed model, Tukey; $P < 0.05$). Values are mean + SD ($N = 4$).

Table 4
Results of modelling MOULDER (α-coefficient), water stable aggregates (WSA) or SlakeLight (SAS_{trans}) depending on fixed effects total abundance of earthworms (W), soil tillage (T) and levels of earthworm-processed soil (L: bulk soil; burrow-midden-complex) at site 2. *F*-values and *P*-values, denominator degrees of freedom (DenDf).

Dependent parameter	Reduced model	DenDf	*F* – value	*P* – value
α-coefficient	L : W	60	4.47	0.039
	T : W	121	3.39	0.037
WSA	L : W	81	30.2	< 0.001
	L : T	82	28.7	< 0.001
	T : W	83	9.86	< 0.001
SAS_{trans}	L : W	32	4.37	0.045

harvest in early November 2019 or 2020. Hence, differences between sites for WSA and SlakeLight were related to annual weather conditions rather than crop rotation (Rieke et al., 2022) or soil properties, while MOULDER was unaffected.

4.2. Earthworms and soil aggregate stability

Total abundance of earthworms decreases with soil tillage intensity (Briones and Schmidt, 2017), and in the current study this was seen for bulk soil, but not for the burrow-midden-complex. Most earthworms were found around burrows of *L. terrestris* and only half as many earthworms found in bulk soil. Overall, the earthworm community comprised mainly of endogeic and juvenile earthworms. Similar patterns were found by Butt and Lowe (2007) at a woodland site and by Stroud et al. (2022) in arable fields in the UK, when total abundance in the burrow-midden-complex was 1.8–6 times higher or 1.5 times higher than in bulk soil, respectively. In addition, Butt and Lowe (2007) associated eight species to burrows, while in the current study only four species including *L. terrestris* were identified. *Aporrectodea caliginosa* was the most prominent species in all levels, while *L. terrestris* and anecic juveniles were in low numbers mostly in middens and the burrow-midden-complex. This was also seen by Butt and Lowe (2007), and by Euteneuer et al. (2020) in a previous field experiment at site 2. The small numbers of anecic earthworms were a result of the hand-sampling method. Using a suspension of mustard powder and water can produce higher numbers of anecic earthworms (Butt and Grigoropoulou, 2010), but hand-searching was conducted due to the low infiltration rate of the soil. The Chernozem soil at site 2 is known for its high silt content and erodibility under ploughing conditions (Weninger et al., 2019), which can block infiltration of a mustard suspension and reduce the sampling rate. However, the findings of the current study support the importance of the burrow-midden-complex of *L. terrestris* as habitat for endogeic earthworms (Butt and Lowe, 2007) and as hotspots for endogeic earthworm, *L. terrestris* and, as seen by Stroud et al. (2016, 2022), for soil meso- and microfauna too.

Results showed that soil aggregate stability depended on earthworm abundance, processed soil level and/or soil tillage as reported by many other authors (e.g., Ketterings et al., 1997; Jégou et al., 2001; Lehmann et al., 2017; Arai et al., 2018). Middens were especially more stable compared to bulk soil for MOULDER, but not always for WSA and SlakeLight. However, Hallam and Hodson (2020) found that *L. terrestris*

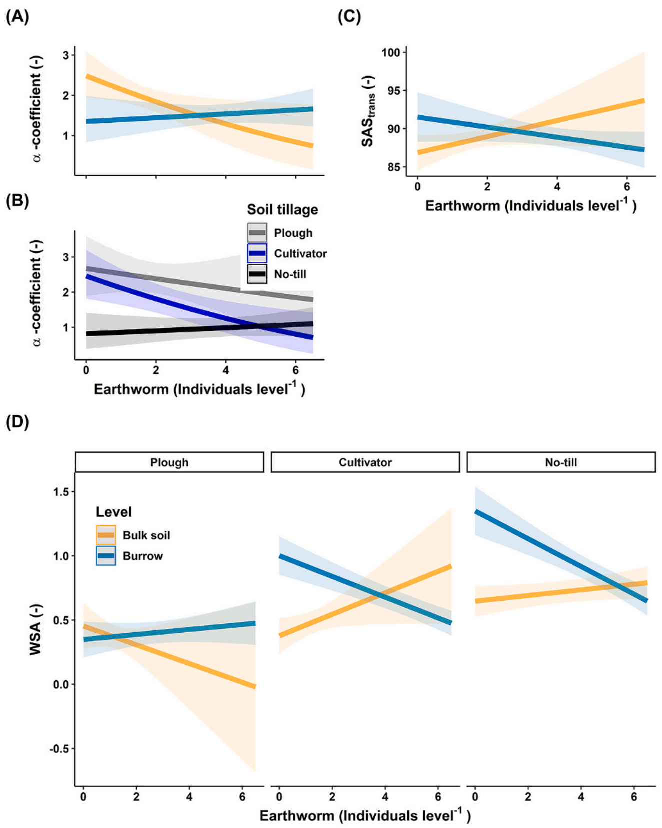

Fig. 5. Fit of MOULDER (α-coefficient; A, B), SlakeLight (SAS_{trans}; C) or water stable aggregates (WSA; D) as function of total earthworm abundance by degree of earthworm-processed soil levels across soil tillage systems (A, C) or as function of soil tillage systems across earthworm-processed soil levels (B) at study site 2. Details of regression estimates are given in Supplementary Table S1. Values are mean, 95 % confident interval ($N = 4$; shaded area). Note: α-coefficient has an inverse relationship with WSA and SAS_{trans} and is square root transformed.

increased stability by 16–56 % relative to an earthworm-free control, mostly in the first 6.5 cm of soil where cast was more present, similar to middens. This finding also applied for *A. chlorotica*, which showed a similar increase (19–63 %) in the upper soil layer but contrasts with the current results. In the current study, the burrow-midden-complex and bulk soil showed similar aggregate stability independent of soil tillage or method used. This could be explained by a 3-times lower earthworm density than that of Hallam and Hodson (2020), who used 12 *A. chlorotica* in a mesocosm experiment of 6 cm in diameter and 13 cm height. In addition, for the current study it is not known how many endogeic earthworms lived within the burrow-midden-complex in the field, and this might explain why this level was not distinctly different from bulk soil for all methods according to 3-way LMM. Conversely, Stroud et al. (2022) measured higher soil aggregate stability under middens (5–15 cm diameter; 5 cm depth) by using the rapid wetting test (Le Bissonnais, 2016). Given the two studies of Hallam and Hodson (2020) and Stroud et al. (2022), the sample depth for soil aggregates might sensibly be reduced to 5 cm, instead of 10 cm, to show possible differences between bulk soil and the burrow-midden-complex. In addition, matching the diameter according to the actual midden size seems plausible, but the effect of the soil tillage systems might have been underrepresented.

Due to our findings, we cannot confirm our hypothesis that soil aggregate stability follows a gradient of midden > burrow-midden-complex > bulk soil. However, we saw a relationship between level of earthworm activity, but this was affected by soil tillage and resulted in different degrees of soil aggregate stabilities. Overall, regressions showed a tendency of increased soil aggregate stability with higher earthworm abundance for all methods. This was significant for bulk soil compared to the burrow-midden-complex for all methods but differed between soil tillage systems. For soil tillage, MOULDER found an increase of aggregate stability per earthworm for plough compared to no-till, WSA and SlakeLight for no-till or cultivator compared to plough, but significant interactions of level × soil tillage were only seen for WSA. These interactions showed that the burrow-midden-complex in plough gained higher aggregate stability per earthworm and decreased in cultivator or no-till. Comparable results were seen by Schrader and Zhang (1997), when they stated that the stabilisation of soil aggregate is more effective for soil vulnerable to physical disturbance such as ploughed fields. In addition, Bottinelli et al. (2017) observed that endogeic earthworms increased aggregate stability due to foraging in soil with low organic C content. This is supported by the lower SOC of 2.3 for plough (0–10 cm) than cultivator (2.5) or no-till (2.7) measured in 2020 by Liebhard et al. (2022). This also accords with Hallam and Hodson (2020), who reported a larger relative increase for soil with lower organic C content, but an overall higher stability for soils with high organic C content.

4.3. Method evaluation

WSA is more sensitive to changes of soil tillage and earthworm-processed soil by earthworm abundance, but less sensitive to earthworm cast in middens. For MOULDER, middens were always more stable than soil with less earthworm cast, such as burrow-midden-complex or bulk soil. Overall, MOULDER and SlakeLight showed differences for no-till compared to plough. In addition, MOULDER had the highest coefficient of variance followed by WSA > SlakeLight, which was influenced by whether there were very large (MOULDER) or very small (SlakeLight) differences between levels of earthworm-processed soil. Therefore, like Rieke et al. (2022), the within-treatment variability was relevant and showed that results of MOULDER were shifted towards middens and earthworm cast. In contrast to Rieke et al. (2022), WSA was more elucidating with regards to interactions of earthworms and soil tillage, but also secondary aspects such as the specific research question, experimental set-up, and resource availability need to be considered to select a method. For example, citizen science projects, could gain from MOULDER due to its ease of handling, set-up, and requirements (smartphone (Android or iOS), deionised water and light sources). MOULDER was able to identify earthworm-processed soil or soil tillage systems but failed to show any interactions. Both WSA and SlakeLight need trained staff and scientific equipment, while MOULDER can be used by lay people. A disadvantage of MOULDER is that, unlike WSA or SlakeLight, the α-coefficient is not expressed as a proportion and therefore reference samples of undisturbed soils such as field margins or lawns are needed for farmers to categorise their soil tillage management.

5. Conclusions

A comparison of the methods MOULDER, WSA and SlakeLight showed similar results, but with a different degree of sensitivity to earthworm-processed soil. For soil tillage experiments with earthworm interaction, WSA should be considered, while SlakeLight was able to distinguish between soil tillage systems, but rarely in context with earthworm abundance. MOULDER showed differences for soil tillage and was most responsive to earthworm cast, is also easy to handle and less resource intensive than other methods. However, earthworms increase soil aggregate stability, with the effects of higher earthworm abundance foremost in bulk soil than burrow-midden-complex. While burrows have higher numbers of earthworms than bulk soil, except for no-till. Burrows of *L. terrestris* also showed their importance as habitat for endogeic earthworms and indicate interactions of ecological groups. Due to interaction of *L. terrestris* and endogeic earthworms, it was seen that erodible silty soil was stabilised by the activity of earthworms and the extent to which earthworm can provide ecosystem services.

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CRediT authorship contribution statement

P. Euteneuer: Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **K.R. Butt:** Supervision, Conceptualization. **H. Wagentristl:** Resources, Project administration. **M. Mayerová:** Writing – original draft, Validation, Methodology, Data curation. **M. Fér:** Writing – original draft, Validation, Supervision, Methodology, Data curation.

Declaration of competing interest

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Data availability

Data will be made available on request.

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