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Observations on Earthworm Communities and Soils in Various Natural and Man-Affected Ecosystems

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Abstract: Earthworms are important members of the soil fauna since they regulate fundamental soil functions such as organic matter breakdown, fertility, structure and water balance. However, so far, their community assemblages have been poorly investigated in Greece. In this context, the earthworm communities of forty five sites in Greece, belonging to three types of ecosystems—terrestrial water bodies, undisturbed (natural) sites and cultivated (agricultural and urban) fields—were investigated using the combined method of digging and hand sorting followed by the application of a 0.4% formaldehyde solution. Specific soil parameters and various environmental characteristics were examined as potential factors affecting the abundance and species richness. The results showed no statistically significant difference between ecosystem densities. The species number was significantly different between ecosystems, with the cultivated fields exhibiting richer communities, with a mean of 5.3 ± 0.6 species per site compared to the natural areas with 2.4 ± 0.5 species per site, while the water bodies showed intermediate numbers (3.6 ± 0.5 species per site). Finally, earthworm densities were positively correlated with species number and percentage vegetation cover and negatively with clay. These results may contribute to understanding how different land uses affect earthworm communities.

Keywords: earthworm communities; natural ecosystems; cultivated fields; terrestrial water bodies; environmental factors

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1. Introduction

Earthworm ecology and behavior have long attracted scientific interest due to their important role in soil ecosystems [1–11]. The research of earthworm populations in their natural habitats is a challenge due to the numerous components interfering with the structure of the earthworm communities, such as zoogeographical zone, climate, altitude, topography, water availability, land use, soil properties, type of vegetation, relationships with the natural fauna and human intervention, among others [12]. Characteristic is the recent concentric study [13], which is actually a meta-analysis of the global literature on earthworm communities in relation to soil factors, land use, land management and other parameters.

Very useful information on earthworm communities can be obtained from research dealing with the impacts of different agricultural practices on cultivated soil. In this context, it was concluded [14] that soil organic matter and pH enhance earthworm biodiversity, abundance and biomass in grasslands in Northern France, though sand content and livestock density have negative impacts. The average density was 517 ± 57 individuals m^{-2}

and the annual species richness fluctuated from 11 ± 0.4 to 7.9 ± 0.4, depending on the weather conditions in each year. Moreover, the earthworm communities in Slovenian vineyards [15] were influenced by soil and vegetation management. The best treatments were straw mulching and green cover and the mean densities fluctuated from 178 ± 15 to 66 ± 7 individuals m^{-2}. Finally, in another study in Central England [16], the earthworm densities were determined by the use of land and were very high inside leys. The total mean abundances fluctuated from 472 ± 366 to 185 ± 132 individuals m^{-2} in arable land, depending on the year, and reached 732 ± 244 individuals m^{-2} in the leys.

Data on the Greek earthworm communities are available from faunistic-systematic research dating back to 1832, when Brullé studied the first records from various places of Peloponnese [17]. Prominent taxonomists have examined the Greek earthworm fauna while attempting systematic investigations of the material collected by themselves or brought by other persons. Pioneer work was conducted by the Hungarian Natural History Museum and other institutions in Budapest from the decade of 1980 till the recent years [18–22]. Nevertheless, the taxonomic publications usually give detailed information concerning the regions and the biotopes of collection, which reflect the ecological preferences of the species, but are lacking the numerical data that may describe the populations; the same is true for quantitative measures on the environmental factors, such as soil parameters. Some exceptions are listed: In [23], the authors attempted to interrelate soil factors (pH, organic matter, organic carbon and sand content) with species and species richness in the province of Pella (Macedonia). They found a positive correlation between species richness and soil organic matter and a negative one with percentage of clay and silt. They described earthworm assemblages too. The authors in [24] interrelated the species of Thrace with the geological origin of the soils and classified them into five different categories. The reference density, depth of distribution and seasonal fluctuation of *Allolobophora dofleini* are indicated in another study [25].

Studies on the populations of earthworms in Central Greece (Magnesia prefecture) were conducted by [26], who concluded that organic olive production supported higher species densities, biomass and richness, and enhanced the soil moisture and organic matter compared to the conventional one. The same authors [27] revealed that the fertility of the soils is positively correlated with earthworm densities. In their study, the species richness in olive groves was 2.4 ± 1.18 in the organic fields and 1.6 ± 0.88 in the conventional ones. In a recent study [28], the yield of olive trees was related to various agro-environmental factors, among which is the earthworm density and data on earthworm species occurrence in organic and conventional fields. Other authors [29] presented data on earthworm densities in cultivated soils clustered by crop species and land management, along with the values of certain soil parameters. Almost all the studied areas were distributed around Greece. They recorded a strong positive correlation between densities and organic matter but no significant correlation with soil N values. Finally, data on the influence of the intensity of the soil mechanical cultivation on earthworm densities revealed that, in Greece, similarly to all over the world, the conventional cultivation system is detrimental to earthworms compared to the conservational and the no-tillage ones, under any investigated crop [30].

The present study attempts to investigate the earthworm communities in a variety of areas in Central Greece, classified into three types of ecosystems: water bodies, undisturbed natural sites and man-affected sites (agricultural fields and urban areas). The study also explores the relationships between these communities and a few environmental components, deemed important for earthworm community structure and magnitude. This research may act as a database for the region of Central Greece and it may act as a reference document for similar sites throughout Europe.

2. Materials and Methods

2.1. Study Area

Forty-five sites were surveyed in Central Greece, as shown in the map (Figure 1) between the years 2015 and 2018. Nineteen sites were freshwater sites (springs, rivers, streams and lakes) (Figure 2), 16 were natural areas (along the roads, maquis, groves, forests and semi-woody areas) (Figure 3) and 10 were man-affected plains (cultivated fields, gardens, orchards, etc.) (Figure 4). Therefore, the sampling sites were allocated into three categories of ecosystems: terrestrial water bodies, undisturbed natural areas and cultivated fields, and the data analysis was performed for each category as an entity. The identity of each sampling site (coordinates, species occurrence and analytical results) are available as Supplementary material (Table S1).

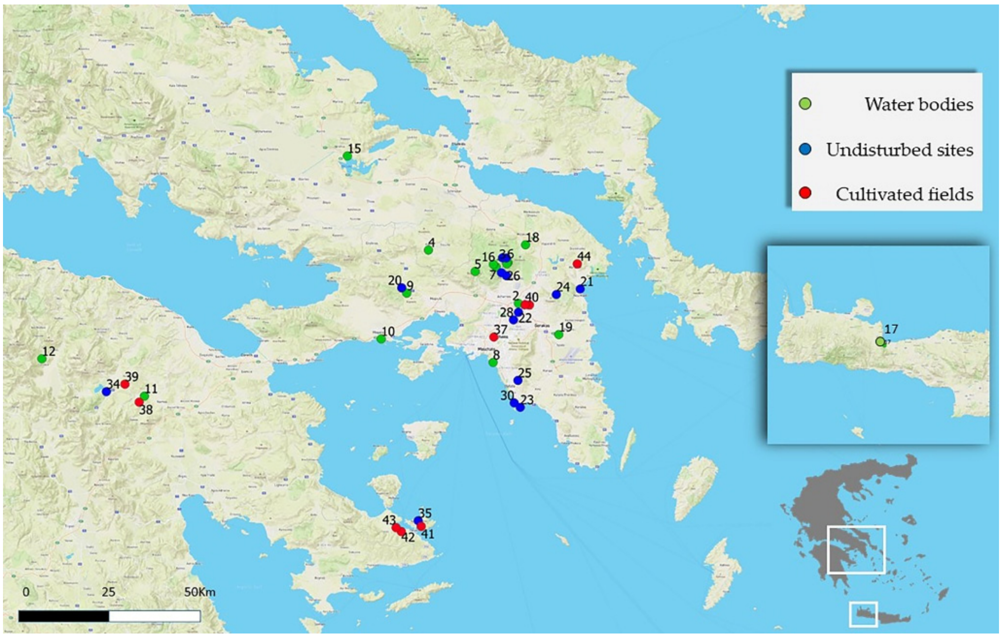

Figure 1. The locations of the study. Water bodies: 1. Menidi river; 2. Karakanta spring; Penteli; 3. Mesiano Nero; Parnitha; 4. Spring Panactos-Oinoi; 5. Filis spring; 6. Kryfo Nero spring; 7. Paliochori spring; 8. Pykrodafni stream; 9. Spring Agia Triada; 10. Pachi stream; 11. Corinthian Asopos; 12. Doxa lake; 13. Sring Koromilia; 14. Spring Kyra; 15. Kifissos; 16. Spring Viliani; 17. Kourna lake; 18. Mpeletsi lake; 19. Spata stream. Undisturbed sites: 20. Menidi road; 21. Nea Makri; 22. Eucalyptos plantation; 23. Faskomilia Vouliagmeni; 24. Dionyssos; 25. Ano Glyfada; 26. Parnitha maquis; 27. Parnitha pineforest; 28. Veikou grove; 29. Parnitha Kamena; 30. Kavouri; 31. Tatoi; 32. Mola; 33. Mola Parnitha; 34. Stymfalia road; 35. Poros pineforest. Cultivated fields: 36. Sygrou; 37. Lawn in AUA; 38. Corinthian vineyard; 39. Corinthian highlands; 40. Marousi; 41. Poros lowlands; 42. Troizina citrus; 43. Troizina olives; 44. Marathon.

Figure 2. A water body: Mountain spring.

Figure 3. A natural site: Maquis of Faskomilia.

Figure 4. An arable field in the Corinthian highlands.

2.2. Sampling and Sorting of Earthworm Samples

In each study site, five samples (replicates) were taken from an area of 33 × 33 m^2, each at the corners and the center of a square, provided that the soil topography was even. Alternatively, the five samples were allocated linearly. At very narrow habitats, such as springs, where only a few meters of soil were homogenous and directly affected by the water, the samples were allocated in smaller distances. Due to the dryness of the climate, the water bodies were preferably sampled very close or on the saturated area. If this was insufficient, the samples were taken, at maximum, 10 m from the water basin and 5 m from the saturated soil. The mixed extraction methodology was adopted [20], with digging and hand sorting up to the depth of 10 cm, followed by application of 4 L of 0.4%. formaldehyde solution. Each sample (replication) covered an area of 0.5 × 0.5 m^2. The earthworms were immediately washed from formaldehyde residues with fresh water, transferred alive to the laboratory and bred until their sexual maturity, when they could be identified to species more safely. During their culturing, they were fed with chopped dry leaves of vine. The identification of the earthworms was performed with the aim of various scientific descriptions and keys [18–20,25,31–35]. In all cases the earthworm densities were calculated as the number of individuals per m^2 ± SE.

2.3. Soil Properties Analyses

For each site some important soil properties were estimated by analyzing a composite sample. Granulometric analysis was performed on the basis of the Bouyoucos method [36]. Soil pH was measured in an aqueous solution (1:1) (pHmeter SELECTA, Spain) [37]. Total soil $CaCO_3$ was measured with the Bernard apparatus [36]. Total soil organic matter was measured using the Walkley–Black chromic acid wet oxidation method [36]. Relative soil moisture (%) was measured as a weight difference after air drying for 7 days at room temperature (Portable, Sartorius AG, Göttingen, Germany). Finally, in each site, data on the vegetation were taken and the percentage of soil coverage by herbaceous plants was estimated, by in situ assessment. The identification of the plants was performed by the staff of the Laboratory of Botany at A.U.A.

2.4. Statistical Analyses

In order to test the homogeneity of the localities within the ecosystems, a Principal Component Analysis and a K-means Cluster Analysis were performed, encompassing all the environmental parameters measured.

The data were subjected to ANOVA and the results are presented as the means ± standard errors. The comparisons of the means were performed using the Tukey HSD criteria with a level of significance of α = 0.05. For the variables that were found to follow a non-normal distribution, after testing by the Shapiro–Wilk W trial, the log transformation of the data to log(X + 1) was used to validate the assumption of normality.

In order to find the determinant factors and reveal the strongest relationships that affect the earthworm communities, bivariate and multivariate analyses were used. The earthworm densities and species number of all sampling sites were correlated with the estimated soil parameters and percentage vegetation cover. In addition, the canonical variates analysis and multiple linear regression were used to capture and determine the relationships between the variables. The statistical analysis was done in JMP 10 [38] and R 4.0 [39].

3. Results

3.1. Earthworm Species and Abundance

In total, 27 earthworm taxa were recorded. From these, 25 were distinguished to species level, one was identified to genus level and for a few juveniles no classification could be done at any taxonomic level. Two of the recorded species, which were non-lumbricid ones, as was concluded by their non-saddle-shaped clitellum, could not been identified further, due to their bad condition after preservation. Most of the species were recorded in very low density to the total sampling. The number of species in the studied sites fluctuated from 0 to 11. The occurrence of each species and the number of species in each site, along with the specific and total densities per site, are presented in Table 1.

Table 1. Earthworm species, mean population densities and the total mean population density in each study area (mean number of individuals m^{-2} ± S.E.).

a/a	Site (Sampling Date)	Type	Species					Total
	Terrestrial Water Bodies							
1	Menidi river, W Attiki (2–8–2015)	river bank	*Ap. c. caliginosa* 44.8 ± 25.5	*B. r.-subrubicundus* 6.4 ± 6.4	*Eis. fetida* 9.6 ± 6.4	*Dendr. veneta* 35.2 ± 11.8		96 ± 46.1
2	Karakanta Penteli Penteli mtn (21–8–2015)	mountain spring	*Ap. c. caliginosa* 0.8 ± 0.8	*Ap. c.–trapezoides* 3.2 ± 2	*O. complanatus* 4 ± 2.5	*Eis. tetraedra* 5.6 ± 3.5	*Dend. b.-byblica* 16 ± 9.6	31.2 ± 15
			unidentified 1.6 ± 1.6					
3	Mesiano Nero Parnitha mtn (23–8–2015)	mountain spring	*Ap. rosea* 4 ± 3.1	*Eis. tetraedra* 4 ± 3.1	*Dendr. b.-byblica* 12 ± 5.9			20 ± 5.9
4	Panaktos –Oinoi North Attiki (1–9–2015)	mountain spring	*Dendr. b.-byblica* 3.2 ± 3.2	unidentified 3.2 ± 3.2				6,4 ± 3.9
5	Filis spring, N. Attiki (13–9–2015)	mountain spring	*Ap. c.- trapezoides* 86.4 ± 36	*Eis. tetraedra* 3.2 ± 2	*Dendr. b.-byblica* 84.8 ± 45.9			177.2 ± 79.3
6	Kryfo Nero, Parnitha mtn (28–10–2015)	mountain spring	*Ap. rosea* 12 ± 5.9	*O. complanatus* 4.8 ± 2.3	*Eis. tetraedra* 3.2 ± 2	*B. r.-rubidus* 1.6 ± 1	*Dendr. b.-byblica* 4 ± 3.1	27.2 ± 7.9
			unidentified 1.6 ± 1.6					
7	Paliochori., Parnitha mtn (14–11–2015)	mountain spring	*Ap. rosea* 2.4 ± 2.4	*O. complanatus* 11.2 ± 3.2	*Eis. fetida* 0.8 ± 0.8	*L. rubellus* 14.4 ± 4.8	*Eis. tetraedra* 18.4 ± 16.5	47.2 ± 13.1

Table 1. *Cont.*

a/a	Site (Sampling Date)	Type	Species					Total
	Terrestrial Water Bodies							
8	Pykrodafnis stream Central Attiki (3–4–2016)	river bank	*Eis. tetraedra* 191.2 ± 129.6	*B. r.-subrubicundus* 100.8 ± 79.1				292 ± 208.4
9	Agia Triada Parnitha mtn (7–5–2016)	mountain spring	*Ap. c. caliginosa* 6.4 ± 3	*Ap. c.- trapezoides* 5.6 ± 4.7	*O. complanatus* 2.4 ± 1.6	*Eis. fetida* 0.8 ± 0.8	*All. chlorotica* 4.8 ± 3.9	44.8 ± 19.2
			Oct. lacteum 2.4 ± 2.4	*Micr. phosphoreus* 11.2 ± 6.8	*Euk. saltensis* 6.4 ± 4.1			
10	Pachi stream., W. Attiki (4–6–2016)	stream bank	*Ap. rosea* 8 ± 4.4	*Ap. c.- trapezoides* 22.4 ± 13.1	*Eis. fetida* 57.6 ± 36.8	*Eis. tetraedra* 47.2 ± 21.4	*B. r.-subrubicundus* 3.2 ± 2	244.8 ± 87.6
			Dendr. veneta 3.2 ± 1.5	*Euk. saltensis* 78.4 ± 31.7	*Ocn. occidentalis* 2.4 ± 1.6	unidentified 20 ± 19		
11	Asopos, Corinth (20–6–2016)	river bank	*Ap. rosea* 2.4 ± 1.6	*Eis. tetraedra* 3.2 ± 2.3	*D. byblica-byblica* 9.6 ± 5.5	*Cr. lacuum* 14.4 ± 13.4		29.6 ± 15.2
12	Doxa lake, Corinth (13–8–2016)	lake	*Eis. tetraedra* 9.6 ± 7.8	*Dendr. b.-byblica* 7.2 ± 7.2	*Cr . lacuum* √			16.8 ± 14.9
13	Koromilia, Parnitha mtn (4–9–2016)	mountain spring	*Ap. rosea* 7.2 ± 7.2	*Ap. c.- trapezoides* 0.8 ± 0.8				11.2 ± 7.5
14	Kyra, Parnitha mtn (2–10–2016)	mountain spring	*Dendr. b.-byblica* 12 ± 4.2					12 ± 4.2
15	Boeotic Kifissos, Boeotia (5–10–2016)	estuary	*Ap. rosea* 32.8 ± 30.8					32.8 ± 30.8
16	Viliani, Parnitha mtn (21–7–2017)	mountain spring	*O. complanatus* 6.4 ± 2.4	*Eis. fetida* 0.8 ± 0.8				7.2 ± 2.9
17	Kourna lake, Crete (30–8–2017)	lake						0
18	Mpeletsi, Parnitha mtn (5–11–2017)	artificial lake	*Ap. c.- trapezoides* 4 ± 2.5	*O. complanatus* 3.2 ± 3.2	*L. rubellus* 0.8 ± 0.8	*Ap. rosea* 0.8 ± 0.8		8.8 ± 5.4
19	Spata stream, N. Attiki (2–8–18)	river	*Ap. c.- trapezoides* 0.8 ± 0.8	*Eis. fetida* 0.8 ± 0.8	*Eis. tetraedra* 16.8 ± 16.8	*Dend. b.-byblica* 4.8 ± 4.8		23.2 ± 23.2
	Undisturbed Sites	Type	Species					Total
20	Menidi road,.W. Attiki (9–8–2015)	edge of street (H_2O-logged)	*Ap. rosea* 19.2 ± 9.3	*Ap. c. caliginosa* 6.4 ± 6.4	*Ap. c.- trapezoides* 34 ± 26.7	*O. complanatus* 6.4 ± 3.9		66 ± 36.5
21	Nea Makri, N. Attiki (27–9–2015)	field	*Ap. rosea* 0.8 ± 0.8	*Ap. c.- trapezoides* 5.6 ± 2.5	*O. complanatus* 12.8 ± 8.7			19.2 ± 9.8
22	Eucalyptus plantation outskirts of Athens (25–10–2015)	forest plantation	*Ap. rosea* 12.8 ± 7.9	*Ap. c. caliginosa* 4 ± 3.1	*Ocn. occidentalis* 0.8 ± 0.8			17.6 ± 7.4
23	Faskomilia, S. Athens (2–11–2015)	maquis	*Ap. rosea* 3.2 ± 3.2	*All. dofleini* √				3.2 ± 3.2
24	Dionysos, N. Attiki (21–11–2015)	coniferous & maquis	*O. complanatus* 2.4 ± 1.6	*L. rubellus* 3.2 ± 2.3	*Dendr. olympiaca* 1.6 ± 1			7.2 ± 4.5
25	Ano Glyfada, S. Athens (6–12–2015)	maquis	*Ap. rosea* 5.6 ± 3.7					5.6 ± 3.7
26	Parnitha maquis (26–12–2015)	maquis	*O. complanatus* 0.8 ± 0.8	unidentified 2.4 ± 2.4				3.2 ± 2.3
27	Parnitha pineforest (27–12–2015)	coniferous forest	*O. complanatus* 1.6 ± 1	*Dendr. attemsi*				1.6 ± 1
28	Veikou grove, Athens (1–1–2016)	grove	*Ap. rosea* 92.8 ± 54.3	*Ap. c. caliginosa* 0.8 ± 0.8	*Ap. c.- trapezoides* 2.4 ± 2.4	*O. complanatus* 2.4 ± 1	*Micr. dubius* 5.6 ± 5.6	104 ± 62.5
29	Kamena Parnitha (9–1–2016)	burned forest	*Ap. rosea* 1.6 ± 1.6	*O. complanatus* 3.2 ± 0.8				3.2 ± 0.8
30	Kavouri, S. Athens (17–1–2016)	pineforest & sea bank	*Ap. rosea* 109.6 ± 106.6					109.6±106.6
31	Tatoi, North Attiki (14–2–2016)	coniferous & maquis	*Ap. rosea* 8 ± 8	*Ap. c. caliginosa* 2.4 ± 2.4	*O. complanatus* 8.8 ± 4.1	unidentified 0.8 ± 0.8		20 ± 8
32	Mola, Parnitha mtn (23–3–2016)	alpine meadow	*O. complanatus* 18.4 ± 8.8	*Mur. minuscula* 1.6 ± 1.6				20 ± 8
33	Mola-Parnitha (13–4–2017)	alpine meadow	*Ap. rosea* 41.6 ± 24.7	*O. complanatus* 4 ± 2.2	*Micr.phosphoreus*			45.6 ± 24
34	Stymfalia road, Corinth (7–8–2016)	road alley	*Ap. rosea* 27.2 ± 17	*O. complanatus* 8 ± 3.3				35.2 ± 18.9
35	Poros island, pineforest (3–12–2016)	coniferous forest						0
	Cultivated Fields	Type	Species					Total

Table 1. *Cont.*

a/a	Site (Sampling Date)	Type	Species					Total
	Terrestrial Water Bodies							
36	Syngrou field, N. Athens (4–10–2015)	garden	*Ap. c. caliginosa* 0.8 ± 0.8	*O. complanatus* 9.6 ± 6.8	*L. rubellus* 2.4 ± 2.4			12.8 ± 9.9
37	Lawn in AUA West Athens (30–6–2016)	public garden	*Ap. rosea* 367.2 ± 51.5	*Ap. c. caliginosa* 80.8 ± 31.9	*Ap. c.- trapezoides* 80.8 ± 31.9	*O. complanatus* 0.8 ± 0.8	*Eis. fetida* 0.8 ± 0.8	673.6 ± 158.7
			Euk. saltensis 2.4 ± 2.4	*A. chlorotica* 53.6 ± 47.8	*Am. gracilis* 2.4 ± 2.4	*Unidentified sp.1* 134.4 ± 56.9	*Unidentified sp.2* 8.8 ± 8.8	
38	Vineyard, Corinth (24–9–2016)	vineyard	*Ap. rosea* 7.2 ± 7.2	*Apporectodea spp.* 3.2 ± 3.2	*Dendr. olympiaca* 0.8 ± 0.8			11.2 ± 10.2
39	Corinthia highland Corinth (30–10–2016)	fallow field	*Ap. rosea* 24 ± 13.1	*Ap. c. caliginosa* 4.8 ± 4.8	*O. complanatus* 5.6 ± 3	*L. rubellus* 3.2 ± 2.3	*Dendr. olympiaca* 0.8 ± 0.8	48.8 ± 22.2
			Micr.phosphoreus	*Micr. dubius* 4.8 ± 2.9	*Ocn. occidentalis* 5.6 ± 5.6			
40	Marousi, N. Athens (13–11–2016)	garden	*Ap. rosea* 2.4 ± 1.6	*Ap. c.- trapezoides* 0.8 ± 0.8	*O. complanatus* 18.4 ± 6.4	*L. rubellus* 1.6 ± 1.6		23.2 ± 7.9
41	Poros island, lowlands (3–12–2016)	plains	*Ap. rosea* 8.8 ± 8.8	*Ap. c. caliginosa* 1.6 ± 1.6	*Ap. c.- trapezoides* 2.4 ± 1.6	*O. complanatus* 28.8 ± 12	*L. rubellus* 4 ± 2.5	45.6 ± 23.4
42	Troizina citrus East Peloponnese (4–12–2016)	citrus orchard	*Ap. rosea* 3.2 ± 2	*Ap. c. caliginosa* 0.8 ± 0.8	*Ap. c.- trapezoides* 6.4 ± 3.7	*O. complanatus* 3.2 ± 2		13.6 ± 7.1
43	Troizina olives East Peloponnese (4–12–2016)	olive orchard	*Ap. rosea* 0.8 ± 0.8	*Ap. c. caliginosa* 1.6 ± 1.6	*Ap. c.- trapezoides* 2.4 ± 1.6	*O. complanatus* 12 ± 11		16.8 ± 12.2
44	Marathon, N. Attiki (22–1–2017)	organic vegetables	*Ap. rosea* 9.6 ± 6	*Ap. c. caliginosa* 30.4 ± 22.7	*Ap. c.- trapezoides* 15.2 ± 11.6	*O. complanatus* 2.4 ± 1.1	*Eis. tetraedra* 1.6 ± 1	119.2 ± 24.3
			Micr. phosphoreus 31.2 ± 11.6	*Micr. dubius* 25.6 ± 15.4	unidentified 3.2 ± 2.3			
45	Corinthia highland (22–10–2017)	bushfield	*Ap. caliginosa* 1.6 ± 1.6	*O. complanatus* 2.4 ± 2.4	*L. rubellus* 0.8 ± 0.8	*Ocn. occidentalis* 1.6 ± 1	unidentified 1.6 ± 1	8 ± 6.2

All. chlorotica: Allolobophora chlorotica (Savigny, 1826); *All. dofleini*: Allolobophora dofleini (Ude, 1922); *Am. gracilis*: Amynthas gracilis (Kinberg, 1867); *Ap. c. caliginosa*: Aporrectodea. caliginosa caliginosa (Savigny, 1826); *Ap. c.—trapezoides*: Aporrectodea. caliginosa—trapezoides (Dugès, 1828); *Ap. rosea*: Aporrectodea rosea (Savigny, 1826); *B. r.-rubidus*: Bimastos rubidus-rubidus (Savigny, 1826); *B. r.-subrubicundus*: Bimastos. rubidus-subrubicundus (Savigny, 1826); *Cr. lacuum*: Cryodrilus lacuum Hoffmeister, 1845; *Dendr. attemsi*: Dendrobaena attemsi (Michaelsen, 1902); *Dend. b.-byblica*: Dendrobaena byblica-byblica (Rosa, 1893); *Dendr. olympiaca*: Dendrobaena olympiaca (Michaelsen, 1902); *Dendr. veneta*: Dendrobaena veneta (Rosa, 1886); *Eis. fetida*: Eisenia fetida (Savigny, 1826); *Eis. tetraedra*: Eiseniella tetraedra (Savigny, 1826); *Euk. saltensis*: Eukerria saltensis (Beddard, 1895); *L. rubellus*: Lumbricus rubellus Hoffmeister; *Micr. dubius*: Microscolex dubius (Fletcher, 1887); *Micr. phosphoreus*: Microscolex phosphoreus (Dugès, 1837); *Mur. minuscula*: Murchieona minuscula (Rosa, 1905); *Ocn. occidentalis*: Ocnerodrilus occidentalis Eisen, 1878; *O. complanatus*: Octodrilus complanatus (Dugès, 1828); *Oct. lacteum*: Octolacion lacteum (Örley, 1881).

The mean ecosystem species richness per site is depicted in Figure 5, which differed significantly among ecosystems ($p = 0.0046$).

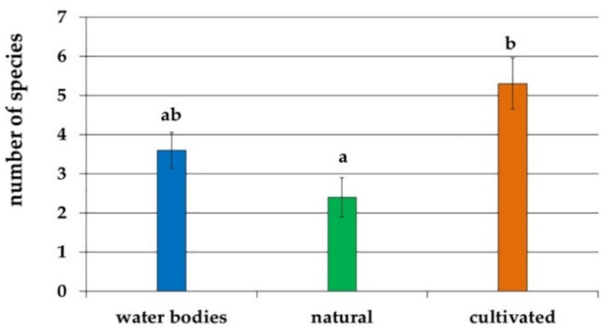

Figure 5. Ecosystem species richness per sampling site. The bars represent the standard errors of the means. Means connected with different letter differed significantly according to ANOVA, followed by Tukey post-hoc test (a,b: denote statistically significant differences between groups).

The richest ecosystem in number of species were the water bodies with 17 species, followed by the cultivated fields, which included 16 species, though only 11 species were counted in the undisturbed sites. Generally, the commonest species were *Aporrectodea rosea* (Figure 6), which appeared in 27 sites, and *Octodrilus complanatus* in 26 sites, followed by *Aporrectodea caliginosa trapezoides* (Figure 7), present in 17 sites. The commonest species in the cultivated fields was *Oc. complanatus*, and in the undisturbed sites both *Ap. rosea* and *Oc. complanatus*; in the water bodies, *Eiseniella tetraedra* and *Dendrobaena byblica-byblica* (Figure 8) were equally the commonest.

Figure 6. The species *Aporrectodea rosea*.

Figure 7. The suspecies *Aporrectodea caliginosa trapezoides*.

Figure 8. The subspecies *Dendrobaena byblica byblica*.

Certain species were confined in just one ecosystem (Table 1). These were *Bimastos rubidus rubidus*, *Bimastos rubidus subrubicundus*, *Dendrobaena byblica byblica*, *Cryodrilus lacuum* and *Octolasion lacteum* in the water bodies; Allolobophora dofleini, Dendrobaena attemsi and Murchieona minuscula in the natural soils; and *Amynthas gracilis*, *Unidentified sp.1* and *Unidentified sp.2* in the cultivated soils. Rare species, which were found in one single locality, were the following: *B. rubidus rubidus* and *O. lacteum*, which were recorded from one water body; *Allolobophora dofleini*, *Dendrobaena attemsi* and *Murchieona minuscula*, present in one natural place; and *A. gracilis*, *Unidentified sp.1* and *Unidentified sp.2*, which occurred in one cultivated soil. The acidophilous species *Dendrobaena attemsii* was present in a single locality in a mountainous forest site.

The three mean ecosystem densities, which are presented in Figure 9, varied a lot, but no significant difference was noted (p = 0.3237). The mean earthworm densities reached 97.3 ± 35 individuals m^{-2} in the cultivated fields, 59.2 ± 25 individuals m^{-2} in the water bodies and 28.9 ± 28 individuals m^{-2} in the undisturbed sites. The mean specific population densities per place had low values in each ecosystem, except for a few species. The species with the highest population densities per site were *Ap. rosea* in undisturbed (20 ± 7.9 individuals m^{-2}) and cultivated fields (41.4 ± 18.8 individuals m^{-2}) and *Eiseniella tetraedra* (16 ± 7.7 individuals m^{-2}) in water bodies. In certain sites some species attained vigorous densities (367.2 ± 51.5 specimens m^{-2} of *Ap. rosea* in 'lawn in A.U.A.' and 191.2 ± 129.6 specimens m^{-2} of *Eis. tetraedra* in the Pykrodafnis stream) (Table 1). Two water bodies obtained unexpectedly high densities, as is shown in Table 1, reaching 292 ± 208.4 specimens m^{-2} at the Pykrodafnis stream and 244.8 ± 87.6 specimens m^{-2} at Pachi stream. An unusual high density also was recorded from one cultivated place (lawn in AUA), reaching 673.6 ± 158.7 specimens m^{-2}.

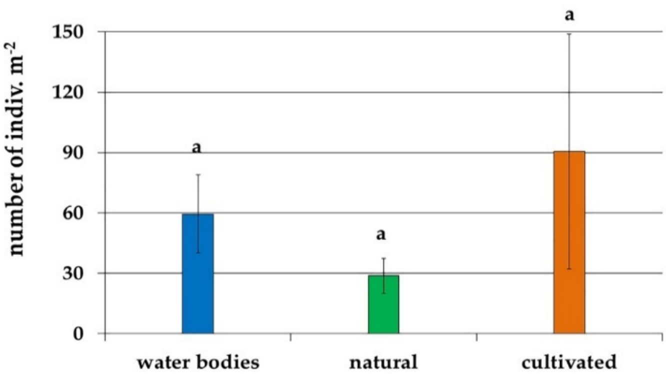

Figure 9. Mean ecosystem densities, along with their standard errors and their statistical comparisons (ANOVA followed by Tukey post-hoc test; a: denote statistically significant differences between groups).

In all undisturbed and cultivated fields, the juveniles were more predominant than the adults regardless of the sampling season. In 5 out of 19 water bodies, the opposite was true.

The earthworm densities were positively correlated with the number of species (r = 0.5730; p = 0.0001) and with the percentage of vegetation cover (r = 0.3376; p = 0.0233). No significant relationships ($-0.2311 < r < 0.2908$) were found between the earthworm parameters and soil properties.

The tests of dimensionality for the canonical multivariate analysis were not statistically significant at the 0.05 level; therefore, multiple linear regression of the response variables of earthworm densities and species number was performed separately with the predictor variables of the soil parameters and percentage vegetation. Forward stepwise variable selection with the minimum corrected Akaike information criterion was used to select the best model. Only the earthworm densities were significantly affected by the percentage of vegetation cover (b_1 = 1.3; R^2 = 0.11; p = 0.0062) and percentage of clay (b_2 = −2.9; R^2 = 0.07; p = 0.0582).

3.2. Soil Properties

The Principal Component Analysis of the environmental parameters rendered a set of three uncorrelated variables. PC1, which explained 33.6% of the variation, was characterized by the positive loading of clay (0.54) and negative loading of sand (−0.59). PC2, which explained 27% of the variation, was characterized by positive loading of pH (0.40) and vegetation cover (0.39) and negative loading of soil moisture (−0.55) and organic matter (−0.53). PC3, which explained 15.1% of the variation, was characterized by a positive loading of $CaCO_3$ (0.60) and loam (0.55). The K-means Cluster Analysis using PC scores divided the regions into three distinct groups (Figure 10) and the results are in accordance with the grouping in the three ecosystems, as described above.

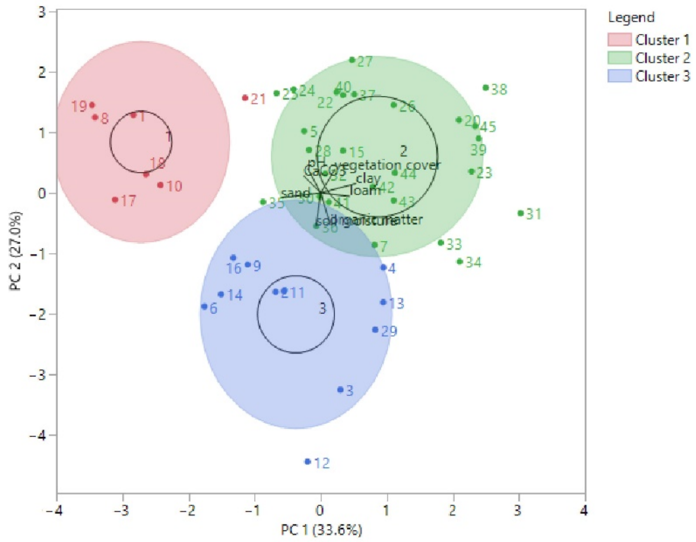

Figure 10. Results of the Principal Component Analysis (PCA) and K-means Cluster Analysis.

Table 2 presents the mean environmental (soil and vegetation) properties estimated in each ecosystem.

Table 2. Some important parameters of the earthworm populations and soils in the three ecosystem types (means ± S.E.), along with their statistical comparisons.

Parameter	Water Bodies	Undisturbed Sites	Cultivated Fields
soil moisture (%)	(29 ± 3) $^{\alpha}$	(17 ± 1) $^{\beta}$	(14 ± 1) $^{\beta}$
sand (%)	(64 ± 3) $^{\alpha}$	(48 ±3) $^{\beta}$	(43 ± 3) $^{\beta}$
loam (%)	(22.5 ± 2) $^{\alpha}$	(29 ± 2) $^{\beta}$	(28 ± 2) $^{\alpha,\beta}$
clay (%)	(13 ± 2) $^{\alpha}$	(24 ± 2) $^{\beta}$	(29 ± 3) $^{\beta}$
pH	(7.4 ± 0.1) $^{\alpha}$	(7.4 ± 0.1) $^{\alpha}$	(7.4 ± 0.1) $^{\alpha}$
$CaCO_3$ (%)	(24.2 ± 4.5) $^{\alpha}$	(22.0 ± 4.9) $^{\alpha}$	(14.3 ± 6.2) $^{\alpha}$
organic matter (%)	(6.0 ± 0.8) $^{\alpha}$	(5.4 ± 0.7) $^{\alpha}$	(4.8 ± 0.9) $^{\alpha}$
vegetation cover (%)	(29 ± 8) $^{\alpha}$	(61 ± 8) $^{\beta}$	(61 ± 10) $^{\beta}$

Different letters in a row denote a statistically significant difference (a = 0.05) between ecosystems, as per ANOVA followed by a Tukey post-hoc test.

The mean ecosystem soils differed significantly from each other regarding moisture content (p = 0.0001), with the water bodies obtaining significantly higher moisture than the other ecosystems (Table 1). Water bodies had a higher mean percentage of sand (p = 0.0001) and lower mean clay content (p = 0.0001) than the remaining ecosystems, as a result of intensive clay loss due to hydrological erosion. They were significantly deficient in loam regarding natural soils (p = 0.0216), though they did not differ from the cultivated soils regarding silt content. The other assessed soil characters (pH, $CaCO_3$ content and organic matter) were equal in all the three ecosystems.

The soil parameters showed a range of significant correlations with each other: soil humidity was negatively correlated with clay percentage (r = −0.3185; p < 0.0330), soil pH (r = −0.3201; p < 0,0320) and percentage vegetation cover (r = −0.3671; p < 0.0131), and positively with soil organic matter (r = 0.5034; p < 0.0004). The sand was negatively correlated with percentage vegetation cover (r = −0.3066; p < 0.0405). Silt was positively correlated with soil organic matter (r = 0.3273; p < 0.0282). Clay was negatively correlated with soil calcium (r = −0.3613; p < 0.0148) and soil pH was positively related with soil calcium (r = 0.4140; p < 0.0047) and negatively with soil organic matter (r = −0.3988; p < 0.0067). The percentages of soil particles were interrelated.

3.3. Vegetation Differences

The mean ecosystem soil coverage by vegetation, as a percentage of the total area, is given in Table 2. Water bodies were estimated with significantly lower vegetation cover than the other ecosystems, which did not differ between each other ($p = 0.0098$). The soil vegetation cover was positively correlated with earthworm densities and negatively with soil moisture and sand content.

There was a big variation among the composition of the plant communities in each place and between the three ecosystems. The commonest species in the terrestrial water bodies were the following: *Cynodon dactylon* L.(Pers.) Poaceae, *Platanus orientalis* L. Platanaceae, *Rubus fruticosus* L. Rosaceae and *Vitex angus-castus* L. Vervenaceae. In the undisturbed sites the frequently recorded plant species were *Cyclamen graecum* Link. Primulaceae, *Cistus* spp. Cistaceae, *Pinus* spp. Pinaceae and *Trifolium repens* L. Fabaceae. Finally, in the cultivated fields the commonest species were *Ficus carica* L. Moraceae, *Oxalis pes-caprae* L. Oxalidaceae, *Piptatherum miliaceum* (L.)Cross Poaceae and *Sinapis* spp. Brassicaceae.

4. Discussion

The lack of significant differences in earthworm densities between ecosystems can be attributed to differences within each ecosystem due to altitude, topography, season, flora, time of intervention from the last rain and soil properties. A more analytical survey might be performed in the future to detect the degree of influence of each parameter.

The cultivated soils showed a tendency to support higher populations compared to the other ecosystems, though this was not significant. This clear trend can be explained by the better nutrition of the plants due to the application of fertilizers and organic amendments as well as to the increased relative humidity levels, due to irrigation, which is capable of prolonging the vegetative and earthworm activities around the year. The use of fertilizers, even the chemical ones, is beneficial to earthworms because it enhances the roots and the microflora in soil and it creates a favorable microclimate due to the presence of plants [1,40,41]. The use of the land for non-intensive agriculture does not harm earthworm species number and densities, as it has been reported by other researchers [42,43], though intensive agriculture is harmful to all soil fauna [44]. Regarding soil mechanical cultivation, which is the rule in agricultural lands or those under manipulation by humans, if it is conservative it can be beneficial to earthworms because it alleviates compaction, favoring aeration and water infiltration and retention [45]. According to [46], non-cultivation of the soil or low-intensity tillage are less destructive to the burrows, do not injure the body, maintain the microclimatic conditions in the drilosphere, blunt the coarseness of the aggregates and do not disrupt the catering of surface resources, being less negative for earthworms compared to intensive tillage. Under an organic system the earthworm densities are usually higher than under conventional methods [45,47]. This was verified with our outcomes from an organic vegetable field in Marathon (Table 1). This observation is in accordance with results in Spain [48], Germany [49] and Greece [26], the latter finding significantly greater values of species richness and density in organic olive groves than in the conventional ones in Central Greece.

According to the literature [8,42,50–52], the best habitats for species number and abundances are the mature broadleaved forests, the grasslands and the pastures, which were not included in our survey. Coniferous forests are very inhospitable for earthworms [53] because they give very recalcitrant litter, which influences the soil quality accordingly. This was not possible to be verified in our survey because the majority of the coniferous forests that were sampled were mostly mixed biotopes, with shrubs and other vegetation.

From the areas included in the water bodies, very high densities were found at two eutrophic streams (the Pykrodafnis stream, an urban brook, and the Pachi stream, a trench accepting water from intensively cultivated fields, as is presented in the data of Table 1), indicating the high tolerance of at least the semi-aquatic species to water pollution. Similar observations were reported in Northern Germany [54]. More sampling should be undertaken in the future at polluted terrestrial aquatic areas in order to reveal specific adaptations.

The commonest species of the total places were *Ap. rosea*, *Oc. complanatus* and *Ap. caliginosa trapezoides*. All of them are K strategists, which implies that they need stable environments to develop [50]. The first one has endogeic habits, living on soil organic matter, so it can cover its food requirements with resources available in most places and all year around. It can tolerate wet conditions, provided that there is O_2 availability [55]. The second is a large anecic species that easily adapts to soils by its rapid movement and by effective exploitation of the available space because the adults live in different soil levels than the juveniles. The juveniles reside near the surface soil, between the roots, and the adults occupy permanent burrows. The third species can change its habits from endogeic to anecic when it confronts hard competition.

According to the literature [1,56,57], the earthworm communities under natural conditions are made up of 1–16 species. In many cases the communities include certain species that coexist in distinct standard combinations. Our results regarding species richness on natural undisturbed sites fell to the lower range of the above researchers (0–5 species per place) and the most plausible reason is the dryness of the Mediterranean climate.

The cultivated fields were richer in total species number, encompassing three imported, non-Lumbricidae (with a ringed-shaped clitellum), considered therefore as allochthonous species—*Amynthas gracilis*, *Unidentified sp.1* and *Unidentified sp.2*—that were exclusively present in this ecosystem. These must have been introduced there with the soil amendments used to improve productivity. In most of the cases, the populations were dominated by juveniles. This is indicative that either they were in an active developmental stage or they seized reproduction in order to aestivate or hibernate. We cannot exclude the possibility that the bigger specimens, such as the adults of many species, escaped into the deeper layers of the soil and avoided capturing, and this could happen in the cultivated and natural soils that normally are deeper than the water bodies. The same developmental stage was prevailing in the surveys of [54] and of [50]. In five water bodies the adult stage overrated the immature one, suggesting that reproduction was intensively accomplished. In most of these places the communities were made up of epigeic species (*D. byblica-byblica, E. tetraedra, Bimastos rubidus rubidus* and *B. rubidus subrubicundus*), which have a relatively short life cycle and survive at the cocoon stage during adverse environmental periods. They follow the r strategy of survival and produce numerous offspring. Presumably, the sampling was done at the time of their preparation to confront the adverse conditions.

There was a strong positive correlation between species number and density, as was expected. Two additional statistically significant relationships between earthworm parameters and their environment were revealed during multiple linear regression, between density and percentage vegetation soil coverage as well as between density and percentage of soil clay. The fact that plants significantly affected earthworms positively is not surprising because they are involved in many important soil processes: they provide earthworms with food in the form of residues and excrements; they regulate some decisive components of the soil systems, such as microclimate and water balance; they utilize the nutrients and resources; and they play a serious role on another soil biota [58,59]. From the other hand, soil clay is associated with high soil fertility and water-holding capacity, but is responsible for high mechanical strength, low aeration and low percentage of extractable water, too. Soil organic matter decomposition is a slow process in heavy-clayed soils due to low O_2 availability and temperature. The earthworms that live in clayed soils put high effort into constructing their burrows and face difficulties in their movement. Taking into account the above, it is not surprising that clay and densities were negatively correlated.

As was expected, the soils of the water bodies obtained significantly higher moisture than the rest ecosystems, though the natural sites had an insignificantly higher value compared with the cultivated soils (Table 2). This can be interpreted by unhindered evaporation of the mechanically disturbed soils of the latter ecosystem and the deeper penetration of the water in the grooved land. The negative significant correlation of the soil moisture with clay content possibly reflects the low clay content of the aquatic ecosystem and this with soil pH comes as a result of the dilution of the CO_2 of the soil atmosphere when the moisture is increased as well as of the negative surface charges when sufficient hygroscopic humus is present. A significant positive correlation of soil moisture with soil organic matter is expected due to the increased ability of humus to retain water. The significant negative correlation with percentage vegetation cover is due to the higher losses of water through increased transpiration of the developed canopy compared to the fallow soil.

Earthworms, like all soil organisms, are very sensitive to soil pH. Their range of tolerance is between 3.1 and 9 [60], but most species can live only in narrower limits, at pH 5–8.5. Each species has its specific tolerance limits. The most acid-tolerant species of the study was *D. attemsii*, which was recorded only in a fir forest with slightly acidic soil (pH = 6.6) and zero $CaCO_3$ (Table 1).

No significant differences were detected between ecosystems regarding Ca content (Table 2), though the cultivated soils obtained a lower value than the other ecosystems, obviously due to its removal at harvesting. Ca is very important for earthworms because it regulates the soil pH to neutral or slightly alkaline levels, which are preferable for most species, and as an essential nutritive element, it promotes the production of fresh plant organs. As a result, more labile tissues are added to the soil [53]. $CaCO_3$ was significantly correlated with soil pH and clay content, as discussed in previous paragraphs.

Among the ecosystems, the cultivated fields had the lowest mean organic matter, although the differences were not significant (Table 2). This is expected as a result of the weed control, the organic matter removal through harvesting and the accelerated soil respiration due to better aeration. In the natural soils the organic matter is transiently protected into the earthworm casts [61] and in the aquatic environment the superficial activity of the dominant epigeic earthworms retain a high level of organic matter close to the surface. The three mean ecosystem values were not significantly different possibly due to the effect of season, but more research is needed to verify the above.

Soil organic matter constitutes the assimilable food of earthworms. When the organic input in the form of autumn-shed leaves, plant residues, organic fertilizers, manure and other nutritive materials are high in a given soil system, the earthworm populations and species number increase [62,63]. The quality of the organic inputs determines the rate of decomposition [64]. A mixture of plant residues, as in crop rotation and the mixed forests, is more balanced and is recycled more readily [65,66], and this signifies the important role of plant biodiversity in environmental sustainability.

Unlike the literature [56,62,63,67] the soil organic matter was not significantly correlated with earthworm densities and species richness. Possible reasons are the diversification of the soil and earthworm community in each site and the fluctuations in soil organic matter related to the season. More research is needed to answer this question.

5. Conclusions

There is big variation between and within ecosystems regarding earthworm species occurrence and richness. In the water bodies some exclusive semi-aquatic species are included, indigenous and cosmopolitan ones, of which few were present in a single locality. Many native species were found in the natural biotopes, among which some rarely occurred. In the cultivated fields, the species richness is high, and many imported, allochthonous species can be found exclusively. The earthworm abundance did not differ significantly between ecosystems. It was obvious that good agricultural practice supports a high earthworm population. Unexpectedly, high densities were obtained from water bodies that suffer from pollution of urban or agricultural origin.

Earthworm species number and densities are positively intercorrelated. The most significant environmental parameters that influence the earthworm density are the vegetation cover of the soil (positively) and the soil clay content (negatively).

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/soilsystems5040071/s1, Table S1: Analytical characteristics of the localities of the survey.

Author Contributions: Conceptualization, E.V. and C.E.; methodology, C.C. (Csaba Csuzdi), E.V. and C.C. (Christina Chalkia); software, A.K.; validation, C.C. (Csaba Csuzdi) and E.V.; formal analysis, C.C. (Christina Chalkia) and A.K.; investigation, C.C. (Christina Chalkia); resources, C.C. (Christina Chalkia) and C.E.; data curation, C.C. (Christina Chalkia), C.E. and A.K.; writing—original draft preparation, C.C. (Christina Chalkia); writing—review and editing, E.V., C.C. (Csaba Csuzdi), C.E. and A.D.; visualization, C.C. (Christina Chalkia), E.V. and A.D.; supervision, E.V. All authors have read and agreed to the published version of the manuscript.

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