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Effect of reduced tillage and conservation agriculture systems on earthworm and microarthropod populations assessed by conventional methods and by metabarcoding

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1 Dansk resumé: Metabarcoding af jordbundsfaunaen i de pløjefri dyrkningssystemer i Aulum og Jerslev

INDLEDNING

Jordbundens sundhed kan bestemmes ud fra jordbundslivet. Jordbundsorganismernes krav til levested og føde i og på jorden gør, at de reagerer på jordens beskaffenhed og de vil derfor afspejle kvaliteten af jorden. En sund jord er således at opfatte som et levested for mange jordbundsorganismer, både antal og diversitet, hvor deres aktiviteter medvirker til god krummestruktur, frigivelse af plantenæringsstoffer, dræning og beskyttelse mod planternes skadegørere.

Der er gennemført mange undersøgelser af virkningerne af reduceret jordbearbejdning på jordens biodiversitet siden 1960'erne, men der findes kun få undersøgelser af den nyere dyrkningsform Conservation Agriculture (CA) praktiseret i overensstemmelse med FAO's retningslinjer (FAO, 2015). I mellemtiden har NGS, Next Generation Sequencing, muliggjort en ny tilgang til vurdering af biodiversitet, hvor man er ved at skifte fra den Linnéske taksonomi, taksonomi version 1.0 baseret på morfologi, til taksonomi version 2.0 baseret på DNA-sekvensering.

I denne rapport har vi udført undersøgelser af jordlevende hvirveldyr, collemboler, mider og regnorme i landbrugsjord med langvarige kontrollerede forsøg med reduceret dyrkningspraksis. Vores mål er at sammenligne den konventionelle vurdering af biodiversitet for hvirvelløse jordbundsdyr med metabarcoding og evaluere udfaldet med hensyn til dets fortolkningsevne og deres overensstemmelse.

Den nuværende måde at analysere DNA biblioteker på opnået via metabarcoding er baseret på mængden af DNA opformeret med PCR af DNA ekstraheret fra jord eller fra en blanding af arter. Herudfra produceres OTU'er, operationelle taksonomiske enheder, som i den bioinformatiske analyse tildeles et artsnavn ved at sammenholde med referencebiblioteker. Metabarcoding leverer ikke biomasse eller densitetsresultater, som de konventionelle metoder, men i stedet et antal DNA strenge for hver OTU.

FORSØGSLOKALITETER

Jordprøver er indsamlet fra de langvarige forsøg med reduceret jordbearbejdning i Aulum, 17 år gammelt, og Jerslev, 20 år gammelt, på henholdsvis lerblandet sand, JB3, og lerjord, JB6, i oktober 2017 og juni 2018. Oktober 2017 var meget fugtig og juni 2018 var meget tør. I Jerslev er dyrkningssystemet omlagt til *Conservation Agriculture* (CA) over de sidste 4 år, mens der i Aulum er kørt med reduceret jordbearbejdning uden pløjning (*reduced tillage*, RT), men med overfladisk kultivering. Begge steder er referenceleddet pløjet (*conventional tillage*, CT).

KONVENTIONELLE RESULTATER

Faunaen blev indsamlet d. 20. april og 25 okt., 2017, samt 4. juni 2018 i Aulum. I Jerslev indsamledes jordbundsdyrene og jordprøver d. 23 okt. 2017 samt d. 11. juni 2018.

I Jerslev fandtes arten Blå orm, *Octolasion cyaneum*, og Løvregnorm, *Lumbricus castaneus*[1], som begge blev bekræftet ved COI *barcoding*. For at sikre at 16S barcoding referencedatabasen indeholder alle regnormearter blev yderligere 16S for *O. cyaneum* barcoded, for senere at kunne identificere den i DNA ekstraheret fra jord.

Vi fandt seks arter af regnorme: Rosa orm, *A. rosea*, Grøn orm, *A. chlorotica*, Stor grå orm, *A. tuberculata*, Lang orm, *A. longa*, Stor regnorm, *Lumbricus herculeus* (bekræftet ved *barcoding*) og Blå orm, *Octolasion cyanum*, i Jerslev. I Aulum fandt vi også seks arter: Rosa orm, Stub orm (*Dendrodrilus rubidus*), *Lumbricus* sp., efterfølgende bestemt til *L. herculeus* ved *barcoding*, samt Stor grå orm (*A. tuberculata*), Grå orm (*A. caliginosa*) og Mosorm (*Dendrobaena* sp.).

Total regnormeantal var på 203 indv. m^{-2} [147-258] og biomassen på 145 g m^{-2} [92-198] var signifikant højere i CA-systemet sammenlignet med 117 indv. m^{-2} [77-158] og 60 g m^{-2} [32-88] i det konventionelle jordbearbejdningssystem i Jerslev. *A. longa* og *L. herculeus* var de dominerende regnorme med hensyn til frisk biomasse (Fig. 2), og den mindre *A. rosea* var mest talrig. Statistisk signifikante forskelle mellem de to systemer kunne detekteres for voksne *L. herculeus* og voksne *A. tuberculata* med 6-7 gange højere biomasse i CA end RT.

Denne forskel var ikke til stede på Aulum-lokaliteten i oktober 2017 med et samlet regnorme antal på 84 indv. m^{-2} [25-142] og en biomasse 36 g m^{-2} [8-64] lignende og ikke signifikant forskellige i det reducerede jordbearbejdning, RT, system sammenlignet med 57 indv. m^{-2} [27-86] og biomasse 16 g m^{-2} [6-25] i det konventionelle jordbearbejdningssystem. Men igen, *L. herculeus* viste sig at være mere talrig med en faktor syv i det upløjede RT-system og tre gange højere biomasse.

[1] Ingen populationsdata tilgængelige. Bestemt som juvenil *Lumbricus* sp. barcode database reference SBS-315.

Regnormebiomassen i Aulum forsøget

		April 2017		Okt. 2017		Juni 2018	
		CT	RT	CT	RT	CT	RT
L. herculeus	Stor regnorm	4.0	5.6	0	**6.8**	0.3	0.3
Dendrobaena sp.	Mosorm	0	0	0	**0.3**	0	0
D. rubidus	Stuborm	0	0	0.2	0.2	0	0
A. tuberculata adult	Stor grå orm	21	9.0	8.5	18.2	2.0	3.9
A. caliginosa adult	Grå orm	1.7	1.4	0	***5.7***	0	0
A. caliginosa and *tuberculata* juvenile		5.5	11.9	6.2	4.6	5.9	7.5
A. rosea	Rosa orm	1.3	0	0.9	0.0	**2.8**	0
Total		33	28	16	36	11	13

Regnormebiomassen i Jerslev forsøget

		Okt. 2017		Juni 2018	
		CT	CA	CT	CA
L. herculeus	Stor regnorm	6.3	38	1.4	0
A. longa	Lang orm	36	*60	21	*61
O. cyaneum	Blå orm	0	***4.1***	0	0.8
A. tuberculata adult	Stor grå orm	3	***22***	0	3.8
A. tuberculata juv.		0.88	0.2	3	**0.1**
A. rosea	Rosa orm	9	15	3.5	5
A. chlorotica	Grøn orm	4.5	5.8	0.4	1.7
Total		60	***145**	29	*72

Mikroleddyr, springhaler og mider, i 2017

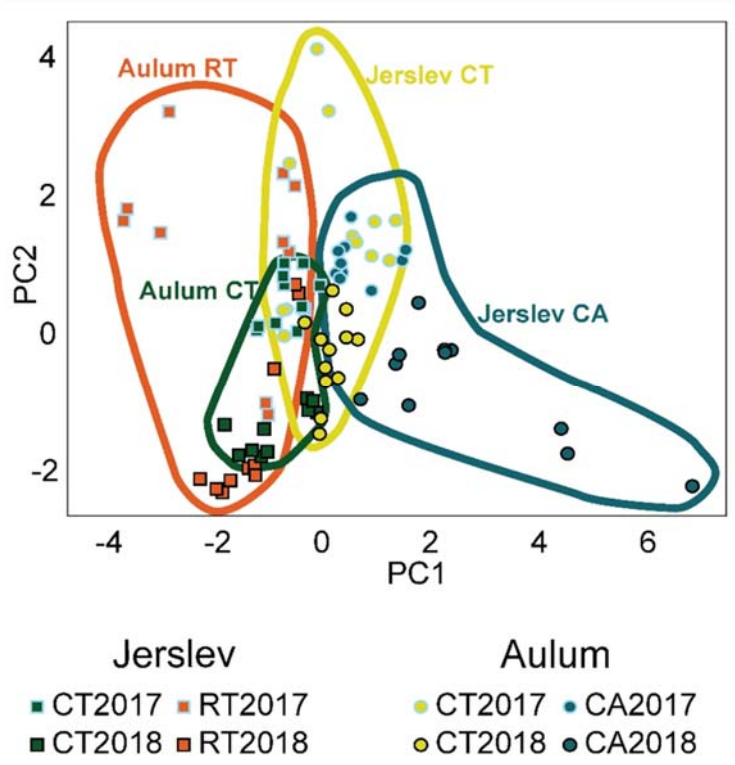
		Aulum		Jerslev	
		CT	RT	CT	CA
Pore-springhaler	Stavhale	4.7	5.0	21.3	11.1
	Firetornstavspringhale	0.4	0.5	0.0	0.1
	Tyk porespringhale	0.0	0.1	4.3	4.4
	Tyk porespringhale			1.5	1.1
Ensledspringhaler	Kvadratøjespringhale	***2.8***	23.0	42.8	33.8
	Almindelig sumpspringhale	3.4	5.3	***7.2***	23.3
	Langhåret fireøjespringhale	***0.6***	3.2	18.3	20.7
	Mark blindspringhale	0.2	0.4	***4.1***	0.7
	Mucrosomia garretti	2.5	2.3		
	Blegspringhale	***0.0***	0.8	0.0	0.3
	Ensledspringhaler	0.1	0.0	0.8	0.5
	Græsspringhale			5.3	9.0
	Langhåret blindspringhale			0.3	0.4
	Folsomides parvulus			0.0	0.1
Butspringhaler	Knibtangspringhaler	0.3	0.3		
	Langhårede tornspringhaler	0.5	1.7	**2.4**	0.0
	Toøjet pygmæspringhale	0.3	0.1	4.0	3.7
	Willemia sp			0.8	0.2
	Kortmundspringhale			0.0	0.1
Kuglespringhaler	*Arrhopalites caecus*	0.7	0.0		
	Kuglespringhaler	0.2	0.4	0.1	0.4
	Firestribet løvkuglespringer	0.0	0.5	0.2	2.8
	Klamrespringere			0.0	0.3
	Bladkuglespringere			2.6	0.8
Neeli-pleona	Almindelig dværgkuglespringer	1.9	0.8	3.0	8.1
Insektspringhaler	(Blå) glansspringhale	0.1	0.3	6.2	0.8
	Punktøjet porcellænspringhale			0.3	1.2
	Sølvspringhale			0.3	0.2
	Pragtspringhale			1.0	1.3
	Porcellænspringhale			0.2	0.7
	Mangebåndspringhale			0.2	0.5
		18.5	44.6	126.8	126.3
Mider	Rovmider	***3.8***	8.7	13.0	9.4
	Pansermider	**1.2**	17.0	19.5	30.6
	Fedtmider	0.4	2.3	1.6	2.9
	Fløjlsmider	7.8	13.5	31.3	23.3
		13.2	41.4	65.4	66.2

METABARCODING RESULTATER

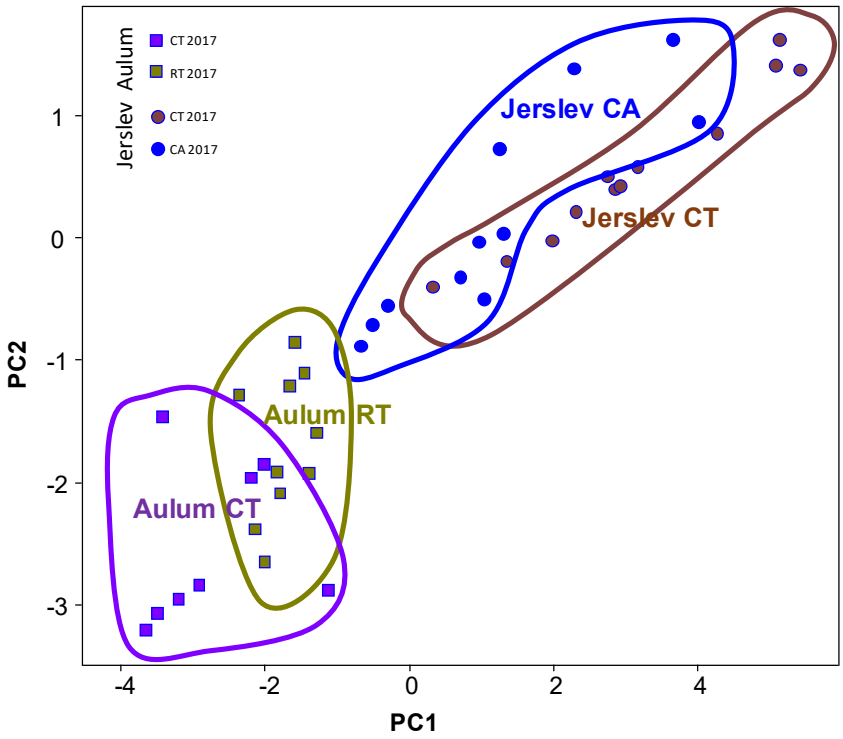
Enkelte regnormearter blev kun fundet i nogle få delprøver med meget lidt DNA. Desuden foreslog den bioinformatiske analyse forekomst af *A. longa*, *A. chlorotica* og *L. rubellus* i Aulum og *A. caliginosa*, *D. octraedra* og *L. rubellus* in Jerslev, men de blev fundet i de håndsorterede jordblokke. Dette kunne skyldes en fejlagtig annotering af artsnavne, men disse arter kunne rent faktisk findes på lokaliteterne uden at blive fundet. Deres andel af DNA *reads* var under 1%. Juvenile *L. rubellus*, Skovregnorm, kan ikke skelnes fra andre *Lumbricus* sp. og er derfor muligvis til stede, men uopdaget.

Ser man på regnormearterne enkeltvis kunne vor metabarcoding ikke skelne mellem dyrkningspraksis i forsøgene i samme udstrækning, som de konventionelle analyser. På trods af dette kan en overordnet multivariat analyse, der tager alle regnorme DNA data i betragtning afdække forskelle mellem lokaliteter og dyrkningspraksis.

Multivariat analyse (PCA) af regnorme DNA ekstraheret fra jord.



Multivariat analyse (PCA) af DNA i jord fra mikroleddyr i 2017



For mikroleddyrs vedkommende ser det ud på samme måde. Der er effekter af dyrkningspraksis ved konventionelt optalte prøver, men ved metabarcoding er disse effekter mest synlige i den multivariate analyse.

Metabarcoding detekterede ca. 25% flere collembolarter end den konventionelle metode, hvor ca. halvdelen af de konventionelt bestemte arter genfandtes ved metabarcoding. En højere kvalitet og kvantitet af DNA kan imødegå denne svaghed. Dette kan opnås ved at bruge længere metabarkoder, så usikkerheden ved tildeling af artsnavne til DNA metabarcoder mindskes. Den konventionelle artsbestemmelse kan føre til fejlbestemmelser og metabarcoding resultatet er det mest pålidelige især ved mange *reads* hvor der er tilstrækkeligt med DNA.

INDIKATORARTER FOR LANDBRUGSJORDENS SUNDHED OG FRUGTBARHED

En landbrugsjord kan miste sin jordbundsstruktur og dermed miste nyttige dyrkningsegenskaber som eksempelvis infiltrationsevne, vandbindende evne og biodiversitet. Dette kan betegnes som en usund tilstand, og begrebet jordbundssundhed kan passende bruges her. Det er således også knyttet til jordens frugtbarhed, da de nævnte egenskaber påvirker landbrugsafgrøden.

Både for regnorme og springhaler er arter knyttet til jordoverfladen indikatorer for gode forhold, mens selve topjordens og pløjelagets populationstætheder afspejler, på sin måde, en god porøs jordstruktur og gode fødebetingelser. For regnormenes vedkommende er de anektiske orme, omfattende Stor regnorm og Lang orm som de vigtigste repræsentanter i Danmark, indikatorarter for gode jordbundsforhold og for springhalernes vedkommende er det de store insektspringhaler, Entomobryidae, og de store ensledspringhaler som Græsspringhalen, *I. anglicana*.

Hvis der alene fokuseres på antal orme vurderes over 100 regnorme pr. m^2 til at være en god bestand svarende til ca. 6 orme på 25x25 cm tælleflade på omdriftsarealer. Antallet svinger hen over året og mellem jordbundstyper og sædskifter osv., så det er et gennemsnit og en tommelfingerregel! Der kan forventes omtrent det dobbelte antal regnorme på et fler-årigt græsareal, dvs. omkring 200 pr. m^2. Ved reduceret jordbearbejdning afhænger antallet af dyrkningspraksis, men man kan forvente væsentligt mere end 100 regnorme pr. m^2.

På en god jord vil der være mange store orme, og på en mindre god jord kan der stadig være mange orme, men de vil være små. Det er dog ikke alene antallet det kommer an på, men også artsammensætningen og biomassen, og det spiller en vigtig rolle hvorledes det indgår i en formel for et index over jordbundskvaliten.

Et biologisk jordbundskvalitetsindex skal afspejle et væsentligt positivt bidrag fra overfladelevende livsformer. Et eksempel på et sådant index er det italienske QBS-e indeks: Qualità Biologica del Suolo og "e" står for *earthworms* (Paoletti *et al.*, 2013; Fusaro *et al.*, 2018). Det er baseret på antal orme pr. m^2 og tilgodeser de anektiske orme, som er Stor regnorm og Lang orm i Danmark, men belønner også en god bestand af de endogæiske orme. Kommer man fra en tilstand med høj grad af forstyrrelse af jordbunden uden anektiske orme, en situation der vil give en score på "dårlig" til "OK", henholdsvis score 1 og 2, kan en øget mænge af anektiske orme på 25 pr. m^2 give en god kvalitet, score på 3, mens der skal et løft til på godt 50 anektiske orme pr. m^2, for at få en maximum score på "fortræffelig", score 4. Skulle jorden kun indeholde endogæiske orme, skal der være omkring 350 af dem pr. m^2 for at få maximum 4 scoren. Dvs. fraværet af anektiske orme skal ikke kunne give maksimum score pga. af mange endogæiske orme, så indekset skal afspejle vort krav til, at der bør være dybdegravende anektiske orme. Dermed vil et nyt indeks også være med til at garantere ormenes bidrag til infiltrationen, som er deres bidrag til økosystemtjenesten dræningskapacitet. QBS-e indekset bør tilpasset nordeuropæiske forhold og i øvrigt tilpasses vore krav til en sund jord og gradueres efter sand eller ler.

HVAD KOSTER DET?

Eksklusiv prøvetagning i felten er prisen for levering af artslister for regnorme og collemboler via rutinemæssig gennemførsel af metabarcoding ca. 750 kr. Prøvetagningen er sammenlignelig med indsamlingen af jord til jordbundstekstur og andre kemiske jordbundsanalyser. Der er for tiden ikke kommercielle eller offentlige laboratorier, der kan gennemføre processen billigere, da der ikke er stordriftsfordele med den nuværende lave efterspørgsel af indlysende grunde, da teknikken ikke er bredt kendt og heller ikke markedsført for invertebrater.

Moderne DNA laboratorier har allerede nogle effektive arbejdsproccesser, der kan håndtere mange prøver på en gang. Det første trin, hvor der bruges arbejdstid på at ekstrahere DNA fra jord, er det mindst udviklede, hvor der er mest brug for optimering.

Prisen for konventionel bestemmelse af biodiversitet for de to grupper er ca. 3 gange, dvs. omkring kr. 2250, dækkende 1 mikroleddyrprøve og 1 regnormeprøve. Beskrivelsen af en mark. Der vil altid være brug mindst tre replikater.

UNDERSTØTTELSE AF HØJ BIODIVERSITET I JORDEN

Hvis der er et lavt antal regnorme noget under 100 pr. m^2 og uden anektiske orme giver det anledning til at anbefale tiltag, der kan forbedre tilstanden. Sådanne tiltag vil også forbedre vilkårene for leddyr. Der er en række dyrkningstiltag som understøtter jordbundslivet og dets økosystemtjenester, idet dyrkningen påvirker to hovedfaktorer af betydning for biodiversiteten i jord nemlig: føden, i form af mængde og kvalitet, samt jordbunden, som levested for dyrene, dvs. "kost og logi":

- Afgrøder med lang vækstperioden så som vinterafgrøder og flerårige afgrøder
- Undgå fræsning med roterende knive.
- Undgå jordbearbejdning, og især når regnormene topper i senforår og efteråret og når jorden er fugtig.
- Efterlad afgrøderester på jordoverfladen og begræns evt. nedmuldning til 5 cm's dybde
- Sikre høj pH på mindt fem.
- Undgå skadelige pesticider der kan have effekter på regnorme, selvom de fleste dog i dag er forbudte.
- Forøg jordens humusindhold

Konklusioner

- De konventionelle metoder viser de kendte forskelle mellem pløjefri og pløjede systemer både i Aulum på sandjord og Jerslev på lerjord: De store anektiske regnorme *L. herculeus* og *A. longa* og de epigæiske orme trives bedst uden pløjning. Mikroleddyrene var positivt påvirkede uden pløjning, men det ekstreme vejr ved prøvetagningerne har skjult nogle af virkningerne af dyrkningspraksis.
- Regnorme kan ikke bestemmes, når de er juvenile og visse arter er kryptiske, dvs. de er morfologisk ens, men er faktisk forskellige, her er metabarcoding i stand til bidrage med det rigtige artsnavn.
- Metabarcoding giver det bedste udtryk for jordbundssundheden, når alle arter bruges i en analyse, mens analyser af de enkelte arter ikke giver et letfortolkeligt billede af dyrkningssystemernes indvirkning på regnorme - og mikroleddyrsamfundene.

REFERENCER

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Effect of reduced tillage and conservation agriculture systems on earthworm and microarthropod populations assessed by conventional methods and by metabarcoding

1 Introduction

While plenty of studies have been done on the effects of reduced tillage on soil biodiversity since the 1960'ies yet only few studies exist of conservation agriculture according to the guidelines by FAO (FAO, 2015). In the meantime, NGS, Next Generation Sequencing, has enabled a new approach to biodiversity assessment moving from the Linnéan taxonomy version 1.0 based on morphology to taxonomy version 2.0 based on DNA sequencing. In this report, we have performed studies of soil invertebrates, collembolans, mites and earthworms in agricultural soil with long-term controlled experiments of reduced tillage practices. Our objective is to compare the conventional assessment of soil invertebrate biodiversity with metabarcoding and evaluate the outcome in terms of its interpretability and their agreement.

2 Methods

2.1 Study location

Study locations were Aulum (lat,lon: 56.2378, 8.8535) and Jerslev (lat,lon: 55.6116, 11.2085).

The study design was a non-randomized complete blocks design with two treatments and three blocks at both locations.

The soil type are clayey sand at Aulum (JB3, 6.5% organic carbon) and heavy clay (JB6, 6.2% organic carbon) at Jerslev (Thomsen and Schjønning, 2011).

2.2 Agronomic information

The Aulum cropping system did not include cover-crops. Cropping sequences since the establishment of the farming systems is listed in 6.

2.3 Sample collection

The fauna was collected on April 20 and October 25, 2017, and June 4, 2018, in Aulum. In Jerslev, the soil animals and soil samples were collected on October 23rd, 2017, and June 11, 2018.

2.3.1 Soil samples for manual sorting of earthworms

In each plot three 25×25×30 cm^2 soil blocks were dug with a spade and hand sorted. Three times during 45 minutes, one liter of mustard powder solution (5-10 g per liter tap water) was poured into the hole to catch deep-burrowing worms. This procedure was performed for a few number of samples, and as it did not recover any earthworms, it was skipped for the remaining samples.

2.3.2 eDNA samples

A sampling area, sub-plot, of 3x3 m^2 was marked in the center of the main plot (see Fig. 1A and B). A soil corer ∅=3 cm was used to take samples of the top soil, depth 0-20 cm. Precautionary measures were taken to avoid cross contamination and equipment were cleaned with 0.05 % sodium hypochlorite. Disposable gloves was changed between sampling of the plots. In total 15 subsamples of 100 g were collected per plot and mixed in a bucket. During transportation samples were kept cool, 0-10 °C. Samples were stored at -20 ˚C until processing in the lab.

2.3.3 DNA extraction

Microarthropods were transferred into Eppendorf tubes before DNA extraction. The DNA was extracted with NucleoSpin® soil kit following the manufacturer's procedure.

Environmental DNA was extracted following the protocol by Taberlet *et al.* (2012). Soil samples were placed at +5 °C for thawing the day before extraction. A Na_2HPO_4 buffer solution of 0.12 M and pH = 9.05 was used instead of a saturated phosphate buffer (0.12 M, pH = 8) to avoid DNA degradation during the extraction process. The soil samples were mixed with equal weight of Na_2HPO_4 solution and followed by 15 min 400 rpm of vigorous shaking to release DNA sorbed to particles. An aliquot of the soil / phosphate buffer mixture were transferred into 2 mL Eppendorf tube and then centrifuged for ten minutes at 10 000 rcf, and 700 µL of the resulting supernatant containing extracellular DNA is recovered for the next extraction steps that were carried out with the NucleoSpin® soil kit without the lysis step. Three extracts were performed for each soil core, leading to 24 DNA extracts for extracellular DNA.

Table 1. Primers and indexing adaptors used for the metabarcodes of microarthropods and earthworms.

	Direction	Name	Sequence
Microarthropods	fw	MiteMinBarF7	5'-CATGCITTYRTIATRATTTTTTTYATAG-3'
	rev	MiteMinBarR4	5'-GGATAHACWGTTCAHCCWGTSCC-3')
	fw	Indexing	5'- TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'
	rev	Indexing	5'- GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'
Earthworms	fw	ewD	5'- ATTCGGTTGGGGCGACC-3';
	rev	ewE	5'- CTGTTATCCCTAAGGTAGCTT-3'
	fw	Indexing	5'- TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'
	rev	Indexing	5'- GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'

2.3.4 Library preparation and sequencing

2.3.4.1 PCR

For microarthropods, PCR amplification was carried out with the COI metabarcode primers MiteMinBarF7/ MiteMinBarR4 (Groot *et al.*, 2016), respectively, and flanked by Illumina indexing adapters (Table 1). PCRs were performed in 25 µL reactions with 1µl template DNA, 2.5 µL of PCR buffer, 2.5 µL of $MgCl_2$, 0.2 µL of 25 nM dNTPs, 0.2 µL of AmpliTaq Gold polymerase (Applied Biosystems), 1.5 µL forward primer, 1.5 µL of reverse primer, 1 µL of 20 mg/ mL bovine serum albumin (BSA, New England Biolabs) and 14.6 µL of PCR-grade H_2O (SigmaAldrich). The amplification was performed under the following conditions: Initial denaturation at 95 ˚C for 10 min, followed by 5 cycles of denaturation at 95 ˚C for 40 s, annealing at 43 ˚C for 40 s, and extension at 72 ˚C for 60 s, then by 35 cycles of denaturation at 95 ˚C for 40 s, annealing at 49 ˚C for 40 s, and

extension at 72 ˚C for 60 s. Cycling was completed at 72 ˚C for 10 min. The correct size PCR products were validated on a 2% agarose gel. PCR products were purified with MinElute PCR purification Kit (Qiagen) according to the manufacturer's protocol.

Environmental DNA extracts were used to amplify earthworm DNA. PCR amplification was carried out with the mitochondrial 16S primer pairs ewD/ewE (Bienert *et al.*, 2012). Forward and reverse primers were flanked by Illumina indexing adapters (Table 1). PCRs were performed in 25 µL reactions with 5 µl template DNA, 12 µL AccuPrime™ SuperMix II (ThermoFisher scientific), 1.25 µL of 10 µM forward primer, 1.5 µL of 10 µM reverse primer, 0.5 µL of 20 mg/ mL bovine serum albumin (BSA, New England Biolabs) and 5 µL of PCR-grade H_2O (SigmaAldrich). The amplification was performed under the following conditions: Initial denaturation at 95 ˚C for 10 min, followed by 38 cycles of denaturation at 95 ˚C for 30 s, annealing at 58 ˚C for 30 s, and extension at 68 ˚C for 60 s. Cycling was completed at 72 ˚C for 10 min. The correct bp length of the PCR products were validated on a 2% agarose gel. PCR products were purified with MinElute PCR purification Kit (Qiagen) according to the manufacturer's protocol.

2.3.4.2 Sequencing

Amplicons were prepared for paired-end sequencing on the Illumina MiSeq platform using the Nextera™ DNA library preparation kit (Illumina, San Diego, CA, USA). Indexes were added by PCRs in a 28 µL system with 12 µL AccuPrime SuperMix II, 2 µL Primer P5, 2µL primer P7, 5 µL PCR products and 7 µL H_2O (SigmaAldrich). The amplification was performed under the following condition: 98 ˚C for 1 min, followed by 13 cycles of denaturation at 98 ˚C for 10 s, annealing at 68˚C for 20 s, and extension at 72 ˚C for 20 s. Cycling was completed at 72 ˚C for 10 min. The PCR products were purified using HighPrep™ PCR (MAGBIO) beads. Libraries were pooled according to the concentration measurement from Qubit®3.0 fluorometer (Thermo Fisher Scientific, Waltham, USA) to 17.5 ng/library. They were sequenced using the MiSeq version 2 reagent kit (Illumina) at Aarhus University, Roskilde.

2.3.5 QA – Quality Assurance

2.3.5.1 Collembolan positive controls for metabarcoding

Three replicates of an artificial mixture of 11 collembolan species with each two adult individuals were produced and went along for sequencing of both batches from Oct. 2017 and June 2018 (Table 2). In 2017, there were nine OTUs with high number of reads, i.e. more than 10 per sample and OTU, and five with low number of reads. For the 2018 mock community there were seven below the threshold of 10 reads and 13 with reads for which only 6 could be assigned to species, mostly because there was no yet references available.

Table 2. Species composition of a collembolan mock community of two adult individuals of 11 species from laboratory cultures from the AU Silkeborg laboratory. The metabarcodes of collembolans indicated by "X" could reliably be assigned a species name after metabarcoding.

Collembolan mock community	Detected	
	2017	2018
Protaphorura tricampata	-	-
Ceratophysella denticulata	X	X
Heteromurus nitidus	-	-
Onychiurus yodai	X	X
Folsomia candida	X	X
Proisotoma minuta	-	-
Mesaphorura macrochaeta	-	-
Folsomia fimetaria	X	X
Sinella curviseta	X	-
Protaphorura fimata	-	-
Hypogastrura assimilis	X	X

2.3.5.2 Metabarcode clustering and species name assignment

Illumina NGS sequencing of a mixture of templates is not as precise as Sanger sequencing performed on an almost pure solution of one DNA template. Thus, assignment of species names are provided along with a specified probability and input into the bioinformatic analysis. E.g. in the case of the earthworm *A. rosea* the mitochondrial 16S barcode of 645 bp employed here as a short metabarcode about 70bp has a K2P variability of 0 to 9% for the currently accessible 20 individuals on GENBANK.

2.3.6 Bioinformatics

2.3.6.1 Reference database

Currently GENBANK include about 100 earthworm species with the mitochondrial 16S barcode.

The sequence quality was checked with FastQC (Andrews, 2010). The bad quality 3'-end was trimmed by Sickle v1.33 (Joshi and Fass, 2011). The paired-end reads merging, primer trimming, de-replication, clustering and *de novo* chimera detection were performed according to Mahe's pipeline (Kopylova *et al.*, 2016; Mahé, 2016) with *vsearch* (Rognes et al., 2016), *cutadapt* and *swarm*. *Cutadapt* (Martin, 2011) setting were: 15% error rate, 2/3 of primer length and the minimum length after primer trimming of 158 bp, 70 bp for microarthropod amplicons and earthworm amplicons respectively. *Swarm* (Kopylova *et al.*, 2016) *d* = -1 were used to cluster the OTUs (Operational Taxonomic Units). Chimera and reads with error rate greater than 0.0002 were filtered out. The parameters are not specified here as the default settings were used.

Microarthropod taxon names were assigned to OTU clusters using two taxonomy prediction algorithms, UTAX (http://drive5.com/utax) (Richardson *et al.*, 2016) and BLAST. The resulting taxonomic identities of each OTU were compared. OTUs that did not target neither mites nor collembolans by both UTAX and BLAST were excluded. We trained the UTAX reference according to the UTAX manual (http://drive5.com/usearch/manual/utax_user_train.html, accessed on April, 2016). We obtained reference sequences from BOLD (http://http://www.boldsystems.org). There were 68,093 sequences for collembolans and 61,683 sequences for mites when BOLD was accessed on April 04, 2016. Sequences were converted to

UTAX compatible format, trimmed with MMB primer, and dereplicated before UTAX training. Mite and collembolan sequences were trained together to increase the complexity of the database and subsequently the accuracy of the training. According to the training report, the cut-off was set to 0.8 to ensure the sensitivity was >50% and the error rate was <5%.

We manually verified the taxonomy annotation of individual OTUs supposed to be mite or collembolan by using the identification function on the BOLD website (Figure 2). We applied the common 3% barcode gap. If the similarity was more than 97% to an identified species name, this species name was kept. Otherwise, the taxon information and BIN number were kept. Sequences that were assigned to the same taxon or belonging to the same BOLD BIN were combined into a single OTU.

Earthworm taxon names were assigned by BLAST with 97% similarity. No threshold was used for number of reads, so one read, i.e. singletons, was the minimum retained for BLASTing.

2.4 Data analysis

2.4.1 Parametric analyses of quantitative data

$Log_{10}(x+1)$ transformed population abundances and biomasses were subject to mixed modelling analysis using PROC MIXED (SAS-Institute-Inc., 2016). The factors included in the model were year and tillage system and the random factors block and its interaction with tillage. The sub-samples within plots and sub-sampling of DNA extracts (technical replicates) were treated as repeated measurements. In cases where one treatment had no worm records the Least Square Means associated t-test for a significant difference from 0 was used (SAS-Institute-Inc., 2016). Rarefaction curves were calculated by *EstimateS* (Colwell, 2013).

3 Results

3.1 Earthworms

3.1.1 Conventional approach

3.1.1.1 Statistical comparison of means

In Jerslev, we found six earthworm species: *A. rosea*, *A. chlorotica*, *A. tuberculata*, *A. longa*, *L. herculeus* (confirmed by barcoding) and *Octolasion cyaneum*. The species *O. cyaneum* and *Lumbricus castaneus*[1], were both confirmed by COI barcoding. In Aulum, we also found six species: *A. rosea*, *Dendrodrilus rubidus*, *Lumbricus* sp., which was *L. herculeus* as confirmed by barcoding, and *A. tuberculata*, *A. caliginosa*, and *Dendrobaena* sp.

In October 2017, a total earthworm abundance of 203 indv. m^{-2} [147-258] and biomass 145 g m^{-2} [92-198] were significantly higher in the CA system compared to 117 indv. m^{-2} [77-158] and 60 g m^{-2} [32-88] in the conventional tillage system in Jerslev. The anecics, *A. longa* and *L. herculeus*, were the dominating earthworms in terms of fresh biomass (Table 5 and Fig. 2) and the smaller *A. rosea* were most abundant. Statistically significant differences between the two systems could be detected for adult *L. herculeus* (F-test tillage*year P=2%) and adult *A. tuberculata* (F-test tillage*date P=7%) having 6-7 times higher biomass in CA than RT. The fresh mass of *A. longa* was twice as high with CA compared to CT (F-test tillage P=4%). In June 2018 a summer drought caused a decrease so *L. herculeus* had almost disappeared from the top-soil while *A. longa* was unaffected (Table 5).

[1] No population density data available as the single juvenile individual selected for barcoding was aimed for determining the cryptic *L. herculeus*, but turned out to be *L. castaneus*.

This difference was not present in the reduced tillage, RT, system at the Aulum location in October 2017 with a total earthworm abundance of 84 indv. m^{-2} [25-142] and a biomass 36 g m^{-2} [8-64] similar and not significantly different compared to 57 indv. m^{-2} [27-86] and biomass 16 g m^{-2} [6-25] in the conventional tillage system (Table 4).

However, again *L. herculeus* proved to be more abundant by a factor seven in the no-tillage RT system and three times higher biomass.

The total abundance and biomass was halved from 2017 to 2018 both in the ploughed and the CA systems (Jerslev) while in the CT/RT system only the RT had a similar reduction by 50% (Table 4 and Table 5).

3.1.1.2 *PCA*

The PCA analysis of fresh mass provides a broad overview of the general trends and structure in the dataset. Tillage treatments were only significant for the Jerslev location and this was true for both the first (PC1) and second principal component scores (PC2) accounting for 30% and 15%, respectively, of the variation in the biomass data.

3.1.2 Metabarcoding

A few earthworm species were found only in a few subsamples and with low number of reads including: *Eiseniella tetraedra* (0.4 [-0.3-1.0], *Lumbricus castaneus* (2.3 [0-4.6]), *L. terrestris* 0.04 [-0.03-0.11] and *Aporrectodea trapezoides* 0.003 [-0.001-0.007] in unit of ×1000 reads and [95% C.L.]. Moreover, the bioinformatic analysis suggested *A. longa, A. chlorotica* and *L. rubellus* in Aulum and *A. caliginosa, D. octraedra* and *L. rubellus* in Jerslev, but they were not encountered in the hand-sorted soil blocks. This could be due to species assignment errors to imprecise metabarcodes, but more importantly these species could actually be resident on the site without being captured. Their read abundance accounts for less than 1% of total reads for each location. Juvenile *L. rubellus*, Skovregnorm, would not be distinguishable from other *Lumbricus* sp. and therefore could be present but undetected. It is an endo-epigeic species, which is not expected to thrive in tillage systems, but as the surrounding system is a no-till system, it may be present in the environment and could colonise the tillage plots in Jerslev.

To ensure that our reference database of 16S included all known species from the locations, we barcoded the 16S of *O. cyaneum*, to enable its subsequent identification in soil DNA extracts. However, it was not detected by metabarcoding.

3.1.3 Comparing earthworm counts with metabarcoding

The ability of conventional population estimation to detect differences between tillage systems is indisputable (Briones and Schmidt, 2017). However, has a PCR amplicon based metabarcoding approach the ability to do the same? To this end, the conventional biomass results in Table 4 and Table 5 and the metabarcoding results of Table 10 and Table 11 are compared in Table 12 with respect to the tendency of a response to the tillage factor. From this summary, it is concluded that metabarcoding does not agree with the conventional assessment of the farming system treatment effect.

To approach the question in another manner PCAs of the biomass and the reads are presented in Fig. 4 and Fig. 5.

3.1.3.1 Analysis of earthworm read abundances

Table 10 and Table 11 presents the read abundances of earthworms.

The structure of the abundance data is revealed by a PCA (Fig. 4). The first principal component (PC1), representing 24% of the variability, and the second principal component (PC2), representing 20% of the variability, were subject to a mixed modelling analysis similar to the conventional samples. *A. chlorotica and A. longa* contributed positively (rightmost dots in Fig. 4) to the PCA scores and *A. tuberculata* contributed negatively to the PCA scores (leftmost dots in Fig. 4) of PC1. At the Aulum location RT differed significantly from CT in 2017 (P<5%) but not in 2018 (PC1), while in Jerslev CA differed from CT in 2018 (P<0.1%) but not in 2017 (PC1). PC2 reflects the between-year differences and significant differences were detected between the two years with differing climate conditions at both locations for both systems (PC2). The Jerslev CA-system was less similar to any of the Aulum systems compared to the Jerslev CT system being more similar to the Aulum systems, i.e. the cluster of Jerslev CA samples did not overlap any of the other clusters (Fig. 4).

3.2 Microarthropods

3.2.1 Conventional approach

We found 44,000 microarthropods m^{-2} and 28 species of Collembola in Jerslev and only 15.000 m^{-2} and 18 species of Collembola in Aulum. A few species densities differed between the systems (Table 6). In the RT system, *P. notabilis* and the gamasid and oribatid mites were more abundant than in the CT. In the CA system, only *I. palustris* reflected the no-tilled CA system. We could not detect effects on the diversity except for the richness being larger in the RT system (Table 8).

The 24 samples per location were close to the asymptote of the rarefaction curve and no additional samples will be needed to cover all the species at the locations, see Fig. 3. However, as there were 12 samples per tillage system, 2-3 rare species per location may not have been captured, nevertheless the species number estimations were not significantly different for sample numbers approx. above five.

In 2018, the collembolans in Jerslev suffered from the drought, by having only 1% collembolans in the top 5 cm of the soil compared to 2017 in the CT system (Table 6 and Table 7). In the Jerslev CA system they were a tenth compared to 2017. In Aulum, collembolans were more abundant in the reduced tillage system in both 2017 and 2018. The soil mites were very numerous in the dry 2018 early summer compared to the very wet 2017 autumn. Both mites and Collembola were more abundant in the CA system compared to CT. In Aulum, the collembolans were twice as abundant in the RT system compared to the CT system. The hemi-edaphic *P. notabilis*, *F. quadrioculata* and *C. denticulata* were the contributors to this difference. In Jerslev, the diversity was significantly lower in the CT system than the CA system (Table 8).

3.2.2 Metabarcoding

Before assigning species names to the OTUs, the OTU list of the 100 most dominant mite and collembolan OTUs from 2017 were subject to a PCA (Fig. 6). Clusters were formed for all groups discriminating between the locations for the PC1 component and indicating the differences between the treatments. Similarly when doing a PCA of the collembolan metabarcoding species list there were location clusters (Fig. 7 and Fig. 8) but to a smaller extent treatment clusters.

3.2.2.1 Comparing metabarcoding and conventional diversity estimates

Metabarcoding provided mostly longer species lists than conventional assessments i.e. about 25% more species based on species with minimum 1 average read per treatment (Table 13 and Table 14). Species in common between the two methods was 50% when comparing the conventional species list with the metabarcoding species list (Table 13 and Table 14). Generally, the reads did not differ significantly between the treatments, CT/RT and CT/CA, for the individual species. However, in a few cases (total thirteen overall

of 88 possible comparisons) there were significant differences, but not necessarily agreeing with the conventional differences between the treatments: 8 of the 13 differences had the same direction as the treatment differences observed with the conventional method.

Diversity metrics did show only few differences between the treatments (Table 9) in contrast to the conventional measures (Table 8). However, the metabarcoding collembolan Shannon diversity for 2017 agreed with the conventional (Table 8), both showing that there was a higher diversity in the CA system compared to the CT system. Moreover, the observed identical levels of diversity for both treatments were largely the same for the two methods.

4 Conclusions

The difference between *L. terrestris* and *A. longa* is emphasized in their response to drought. The surface feeding activity of *L. terrestris* may be persistent but it is absent from the top soil while *A. longa* is still found in the top soil, apparently unaffected by the drought conditions.

The conventional population estimation methods have confirmed the well-known (Briones and Schmidt, 2017) differences between no-till and ploughed systems both in Aulum on sandy soil and on Jerslev on clay soil: The great anecic earthworms *L. herculeus* and *A. longa* and the epigeic earthworms are most abundant in no-till systems. The microarthropods were positively influenced with no-tillage, but the extreme weather conditions during the sampling occasions have hidden some of the effects of the cultivation practices.

Earthworms cannot be identified at the juvenile stage and certain species are cryptic forms can neither be identified morphologically, so metabarcoding is able to contribute the right species name of those cases. E.g., *L. herculeus* is indistinguishable from *L. terrestris*, but they have different habitat preferences and may even be indicators of certain beneficial agricultural soil condition.

Metabarcoding provides the best expression of soil health when whole communities of species are employed in multivariate analyses, while analyses of individual species cannot provide an easy-to-understand picture of the impact of cultivation systems on the earthworms and microarthropod communities. This is undoubtedly linked to the fact that metabarcoding cannot directly reflect abundance and biomass.

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6 Tables

Table 3. Cropping sequence since establishment of the no-tillage experiments.

	Aulum	Jerslev
1999		Winter wheat
2000		Sugar beet
2001		Spring barley
2002	Triticale	Spring barley
2003	Winter barley	Winter wheat
2004	Winter rape	Winter wheat
2005	Winter wheat	Spring barley
2006	Winter barley	Spring barley
2007	Spring barley	Winter rape
2008	Winter barley	Winter wheat
2009	Spring barley	Spring barley
2010	Spring barley	
2011	Winter barley	
2012	Winter rape	
2013	Not harvested	
2014	Spring barley	
2015	Winter wheat	
2016	Spring barley	
2017	Spring barley	
2018	Winter wheat	

Table 4. Mean abundance and fresh weight of earthworms per m^2 and 95% C.L. from the CT/RT systems in Aulum. Bold: sign. difference between the tillage systems P<5%; Bold and italic weakly sign. difference between the tillage systems 10%>P>5%. Life-forms: An: Anecic; Ep: Epigeic; En: Endogeic.

Aulum			April 2017		Oct. 2017		June 2018	
			CT	RT	CT	RT	CT	RT
No. of indv. m^{-2}	*L. herculeus*	An	2.7 [-4.2–9.5]	***11*** [-3–24]	0	**5.3** [-0.8–11]	1.8 [-2.3–5.9]	1.8 [-2.3–5.9]
	Dendrobaena sp.	Ep	0	0	0	**3.6** [-1.9–9]	0	0
	D. rubidus	Ep	0	0	1.8 [-2.3–5.9]	3.6 [-1.9–9]	0	0
	A. caliginosa adult	En	2.7 [-4.2–9.5]	2.7 [-4.2–9.5]	0	***12.4*** [-12–37]	0	0
	A. tuberculata adult	En	24 [10–38]	8 [-1.2–17]	7.1 [-1.8–16]	19.6 [0.3–39]	1.8 [-2.3–5.9]	7.1 [-5.4–20]
	A. caliginosa and *tuberculata* juv. [1]	En	16 [-5.2–37]	29 [2.4–56]	41 [11–71]	39 [2.2–76]	21 [3.9–39]	32 [14–50]
	A. rosea	En	5.3 [-8.4–19]	0	7.1 [-1.8–16]	0	**18** [-1.1–37]	0
	Total		51 [26–75]	51 [31–70]	57 [27–86]	84 [25–142]	43 [24–61]	43 [23–62]
Fresh weight g m^{-2}	*L. herculeus*	An	4.0 [-6.2–14]	5.6 [-5.8–17]	0	**6.8** [-1.6–15]	0.3 [-0.4–1]	0.3 [-0.4–0.9]
	Dendrobaena sp.	Ep	0	0	0	**0.3** [-0.2–0.9]	0	0
	D. rubidus	Ep	0	0	0.2 [-0.2–0.6]	0.2 [-0.1–0.6]	0	0
	A. tuberculata adult	En	21 [1.6–40]	9.0 [-1.8–20]	8.5 [-2.2–19]	18.2 [-0.9–37]	2.0 [-2.6–6.6]	3.9 [-2.2–9.9]
	A. caliginosa adult	En	1.7 [-2.7–6.1]	1.4 [-2.3–5.1]	0	***5.7*** [-5–17]	0	0
	A. caliginosa and *tuberculata* juv.	En	5.5 [-3.8–15]	11.9 [-1–25]	6.2 [1.8–11]	4.6 [0.7–8.5]	5.9 [0.8–11]	7.5 [3.1–12]
	A. rosea	En	1.3 [-2.1–4.8]	0	0.9 [-0.4–2.1]	0.0	**2.8** [0–5.7]	0
	Total		33 [12.6–54]	28 [13–43]	16 [6.1–25]	36 [7.8–64]	11 [5.9–16]	13 [5.5–21]

[1] Juvenile *A. tuberculata* and *A. caliginosa* cannot be separated morphologically. These juveniles are considered to be a mixture of *A. caliginsa* and *A. tuberculata.* The section on metabarcoding, Table 11, supports the assumption of presence of both *A. caliginosa* and *A. tuberculata*.

Table 5. Mean abundance and fresh weight of earthworms per m^2 and 95% C.L. from the CT/CA tillage systems in Jerslev. Bold: sign. difference between the tillage systems P<5%; Bold and italic weakly within year sign. difference between the tillage systems 10%>P>5%. *: sign. effects across both years P<5%. Life-forms: An: Anecic; EnAn: Endo-anecic; En: Endogeic. Species in red not found by metabarcoding.

Jerslev			Oct. 2017		June 2018	
			CT	CA	CT	CA
No. of indv. m^{-2}	*L. herculeus*	An	8.9 [0.0–18]	**34** [8.8–59]	1.8 [-2.3–5.9]	0
	A. longa	EnAn	36 [18–53]	*50 [33–67]	23.0 [14.2–32]	*59 [37–80]
	O. cyaneum	En	0	1.8 [-2.3–5.9]	0	1.8 [-2.3–5.9]
	A. tuberculata adult	En	7.1 [-9.3–24]	***23*** [3.6–43]	0	5.3 [-3.4–14]
	A. tuberculata juv.[2]	En	5.3 [-3.4–14]	1.8 [-2.3–5.9]	7.1 [0.6–14]	1.8 [-2.3–5.9]
	A. rosea	En	43 [21–64]	73 [41–104]	23.0 [-1.6–48]	41.0 [18–64]
	A. chlorotica	En	18 [4.8–31]	20 [7.6–32]	1.8 [-2.3–5.9]	5.3 [-0.8–11]
	Total		117 [77–158]	203 [147–258]	57 [25–88]	114 [77–150]
Fresh weight g m^{-2}	*L. herculeus*	En	6.3 [-1.1–14]	38 [4.6–71]	1.4 [-1.8–4.5]	0
	A. longa	EnAn	36 [12–60]	*60 [34–86]	21 [11–30]	*61 [25–97]
	O. cyaneum	En	0	***4.1*** [-5.3–13]	0	0.8 [-1–2.6]
	A. tuberculata adult	En	3.0 [-3.9–9.8]	***22*** [-0.7–44]	0	3.8 [-2.3–9.9]
	A. tuberculata juv.	En	0.88 [-0.6–2.4]	0.2 [-0.3–0.7]	3.0 [-0.2–6.3]	**0.1** [-0.1–0.4]
	A. rosea	En	9.0 [3.9–14]	15 [8.3–22]	3.5 [-0.4–7.5]	5.0 [1.9–8.1]
	A. chlorotica	En	4.5 [0.5–8.5]	5.8 [1.8–9.8]	0.4 [-0.5–1.2]	1.7 [-0.3–3.6]
	Total		60 [32–88]	***145** [92–198]	29 [17–42]	*72 [37–108]

[2] Juvenile *A. tuberculata* and *A. caliginosa* cannot be separated morphologically, but as no adult *A. caliginosa* was found the juveniles are considered to be *A. tuberculata.* The section on metabarcoding, Table 11, supports the assumption of absence of *A. caliginosa*.

Table 6. Mean number of collembolan species and mite orders per sample with Danish names in 2017. Bold: sign. difference between the tillage systems P<5%; Bold and italic weakly sign. difference between the tillage systems 10%>P>5%. To obtain the abundance per square-meter multiply by 354.

		Aulum		Jerslev	
		CT	RT	CT	CA
Onychiu-ri-dae	Tullbergiinae sp.[1]	4.7	5.0	21.3	11.1
	Stenaphorura quadrispina	0.4	0.5	0.0	0.1
	Onychiurinae sp.	0.0	0.1	4.3	4.4
	Onychiurus cebbenarius			1.5	1.1
Isotomidae	*Parisotoma notabilis*	***2.8***	23.0	42.8	33.8
	Isotomurus palustris	3.4	5.3	***7.2***	23.3
	Folsomia quadrioculata	***0.6***	3.2	18.3	20.7
	Folsomia fimetaria	0.2	0.4	***4.1***	0.7
	Mucrosomia garretti	2.5	2.3		
	Isotomiella minor	***0.0***	0.8	0.0	0.3
	Isotomidae sp.	0.1	0.0	0.8	0.5
	Isotoma anglicana			5.3	9.0
	Folsomia spinosa			0.3	0.4
	Folsomides parvulus			0.0	0.1
Poduridae	*Friesea* sp.	0.3	0.3		
	Ceratophysella denticu-lata	0.5	1.7	**2.4**	0.0
	Micranurida pygmaea	0.3	0.1	4.0	3.7
	Willemia sp.			0.8	0.2
	Brachystomella parvula			0.0	0.1
Symphy-ple-ona	*Arrhopalites caecus*	0.7	0.0		
	Symphypleona sp.	0.2	0.4	0.1	0.4
	Sminthurinus elegans	0.0	0.5	0.2	2.8
	Sminthurididae sp.			0.0	0.3
	Sminthuridae sp.			2.6	0.8
Neeli-pleona	*Megalothorax minimus*	1.9	0.8	3.0	8.1
Entomobryidae	*Lepidocyrtus* sp.	0.1	0.3	6.2	0.8
	Pseudosinella alba			0.3	1.2
	Heteromurus nitidus			0.3	0.2
	Orchesella sp.			1.0	1.3
	Pseudosinella sp.			0.2	0.7
	Entomobrya multifasciata			0.2	0.5
	Total Collembola	18.5	44.6	126.8	126.3

[1] Could be spp. i.e. more than one species in this subfamily.

		Aulum		Jerslev	
		CT	RT	CT	CA
Acari	Gamasida	***3.8***	8.7	13.0	9.4
	Oribatida	**1.2**	17.0	19.5	30.6
	Astigmata	0.4	2.3	1.6	2.9
	Prostigmata	7.8	13.5	31.3	23.3
	Total Acari	**13.2**	41.4	65.4	66.2

Table 7. Mean number of collembolan species and mite orders per sample in 2018. Bold: sign. difference between the tillage systems P<5%; Bold and italic weakly sign. difference between the tillage systems 10%>P>5%. To obtain the abundance per square-meter multiply by 354.

		Aulum		Jerslev	
		CT	RT	CT	CA
Onychiuridae	Tullbergiinae	**0.8**	3.9	0.0	0.3
	Onychiurinae	0.2	0.0	0.0	0.1
	Stenaphorura quadrispina	0.1	0.0		
Isotomidae	*Parisotoma notabilis*	5.4	19.8	0.1	0.3
	Isotomurus palustris	13.1	4.4		
	Folsomia quadrioculata	**0.9**	12.1	**0.1**	2.9
	Folsomia fimetaria	2.0	9.0	0.3	0.3
	Mucrosomia garretti	3.2	3.9		
	Isotoma anglicana			0.1	2.0
	Cryptopygus thermophilus	1.3	0.0		
	Isotomidae	0.3	0.8		
	Folsomides parvulus			0.0	0.1
Poduridae	*Ceratophysella denticulata*	**1.3**	8.3	0.3	0.1
	Friesea sp.	0.3	2.4	0.0	0.0
	Willemia sp.	0.2	2.0	0.1	0.3
	Brachystomella parvula	0.5	0.1		
	Micranurida pygmaea	0.0	0.1	0.1	0.2
Symphypleona	*Symphypleona*	0.2	0.3	0.0	0.2
	Sminthurinus elegans	0.5	0.1	0.0	0.1
	Arrhopalites caecus	0.3	0.2		
Neelipleona	*Megalothorax minimus*	1.8	1.6		
Entomobryidae	*Lepidocyrtus sp.*	2.1	2.9	0.1	0.5
	Entomobrya multifasciata			0.3	2.6
	Pseudosinella alba			0.0	0.1
	Total Collembola	**34.1**	71.8	**1.3**	9.9
Acari	Gamasida	13.8	11.6	3.8	5.3
	Oribatida	**1.3**	14.2	22.8	34.1
	Astigmata	0.3	3.1	**2.3**	7.3
	Prostigmata	185.8	227.9	***88.9***	154.7
	Total Acari	201.2	256.9	**117.8**	201.3

Table 8. Mean per sample diversity information quantities. S: Collembolan species richness; Shannon diversity: $H' = -\sum p_i \log_2 p_i$, where p_i is the proportion of species in a sample to total collembolans; Mean equitability: E: $H'/\log_2 n$, where n is number of collembolan species.

	Aulum		Jerslev	
	CT	RT	CT	CA
2017				
S richness	**4.8**	7.1	12.0	12.0
H diversity	1.6	2.1	2.6	2.6
E equitability	0.71	0.75	0.71	0.74
2018				
S richness	6.3	7.2	**0.83**	3.4
H diversity	1.9	2.2	**0.36**	1.3
E equitability	0.74	0.82	***0.32***	0.63

Table 9. Mean per sample diversity information quantities and 95% confidence limits based on metabarcoding creating OTUs and subsequent assignment of species. S: Collembolan OTU and species richness; Shannon diversity: $H' = -\sum p_i \log_2 p_i$, where p_i is the proportion of species or OTUs in a sample to total collembolans; Mean equitability: E: $H'/\log_2 n$, where n is number of collembolan OTUs or species.

		Mean	95% CLM		95% CLM
A) Common microarthropod OTUs 2017					
		CT		RT	
Aulum	S	29	[22-36]	43	[30-55]
	H	2.0	[1.7-2.2]	1.4	[0.9-1.9]
	E	**0.4**	[0.4-0.5]	0.3	[0.2-0.3]
		CT		CA	
Jerslev	S	**85**	[73-98]	56	[46-67]
	H	1.7	[1.4-2.1]	1.8	[1.4-2.3]
	E	0.3	[0.2-0.3]	0.3	[0.3-0.4]
B) Collembolan OTUs 2017					
		CT		RT	
Aulum	S	13	[8-17]	22	[13-31]
	H	1.2	[0.7-1.6]	0.8	[0.4-1.2]
	E	0.4	[0.2-0.5]	0.2	[0.1-0.2]
		CT		CA	
Jerslev	S	**65**	[54-76]	41	[31-50]
	H	0.9	[0.6-1.1]	1.4	[0.9-2]
	E	0.1	[0.1-0.2]	0.3	[0.2-0.4]
C) Collembolan species 2017					
		CT		RT	
Aulum	S	5.8	[4-7]	7.6	[6-9]
	H	0.7	[0.3-1.1]	0.7	[0.3-1]
	E	0.3	[0.1-0.5]	0.2	[0.1-0.3]
		CT		CA	
Jerslev	S	11	[9-12]	9.9	[8-11]
	H	**0.4**	[0.2-0.5]	1.0	[0.7-1.3]
	E	***0.1***	[0.1-0.2]	0.3	[0.2-0.4]
D) Collembolan species 2018					
		CT		RT	
Aulum	S	11	[7-15]	12	[9-16]
	H	1.0	[0.5-1.4]	0.9	[0.6-1.3]
	E	0.3	[0.2-0.5]	0.3	[0.2-0.3]
		CT		CA	
Jerslev	S	5.4	[4-7]	5.7	[3-8]
	H	0.8	[0.3-1.4]	0.5	[0.1-0.9]
	E	0.4	[0.1-0.6]	0.2	[0.1-0.3]

Table 10. Mean relative DNA read abundances ×1000 and 95% C.L. of earthworms at the Aulum location. Species in red was not found by conventional inventory. Bold and italic weakly within year sign. difference between the tillage systems 10%>P>5%. *: sign. effects across both years P<5%. Life-forms: An: Anecic; EnAn: Endo-anecic; En: Endogeic.

		Oct. 2017		June 2018	
Aulum		CT	RT	CT	RT
Lumbricus herculeus	An	197 [93-302]	***84*** *[20-148]*	157 [31–282]	22 [-6.7–50]
Aporrectodea longa	EnAn	25 [8-41]	16 [4.9-26]	0.3 [0.1–0.6]	15 [-4–34]
Aporrectodea rosea	En	224 [194-253]	**66 [24-109]**	21 [-3.7–45]	20 [-4–45]
Aporrectodea tuberculata	En	373 [225-521]	***615*** *[459-770]*	817 [681–952]	748 [638–858]
Aporrectodea caliginosa	En	162 [12-313]	1.7 [0.4-3.0]	3.3 [0.03–6.6]	***167*** *[51–283]*
Allolobophora chlorotica	En	0.06 [-0.05-0.17]	0	0.07 [-0.02–0.15]	0
Dendrobaena octaedra	Ep	15.5 [5-26]	207 [55-359]	0.1 [0–0.2]	3.6 [-2.2–9.4]
Lumbricus rubellus	Ep	2.56 [0.37-4.7]	1.77 [0.43-3.1]	0.3 [-0.2–0.9]	20 [-6.3–47]
Dendrodrilus rubidus	Ep	0	0.2 [0-0.3]	1.2 [-0.2–2.6]	3.1 [-1.8–8]
Dendrodrilus rubidus rubidus	Ep	0	0.6 [0.1-1.1]		
Dendrodrilus rubidus subrubicundus	Ep	1.03 [-0.84-2.9]	8.5 [2.1-15]		

Table 11. Mean relative DNA read abundances ×1000 and 95% C.L. of earthworms at the Jerslev location. Species in red not found in the conventional inventory. Bold and italic weakly within year sign. difference between the tillage systems 10%>P>5%. *: sign. effects across both years P<5%. Life-forms: An: Anecic; EnAn: Endo-anecic; En: Endogeic.

		Oct. 2017		June 2018	
Jerslev		CT	CA	CT	CA
Lumbricus herculeus	An	248 [141-355]	355 [113-598]	183 [62–304]	72 [18–125]
Aporrectodea longa	EnAn	233 [108-358]	201 [50-353]	125 [38–211]	**669 [442–896]**
Aporrectodea rosea	En	279 [189-368]	440 [234-646]	388 [233–544]	**67 [11–122]**
Aporrectodea tuberculata	En	197 [52-343]	**0.23 [0-0.45]**	193 [14–372]	0.3 [0.1–0.4]
Aporrectodea caliginosa	En	5 [2-8]	**0.11 [0.02-0.2]**	1.5 [0.1–2.9]	0
Allolobophora chlorotica	En	0.55 [-0.44-1.5]	0.73 [0.27-1.2]	6.4 [-4.3–17]	***154*** *[51–258]*
Dendrobaena octaedra	Ep	26 [9-44]	**1.8 [0.9-2.7]**	42 [-4–87]	7.2 [-5.7–20]
Lumbricus rubellus	Ep	11 [-2.4-24]	0.19 [0.03-0.35]	62 [22–101]	28 [0.6–56]
Dendrodrilus rubidus	Ep	0	0	0.03 [0.01–0.06]	0
Dendrodrilus rubidus rubidus	Ep	0	0		
Dendrodrilus rubidus subrubicundus	Ep	0.05 [-0.04-0.15]	0		

Table 12. Response to the tillage treatments, as assessed by the two earthworm assessment methods: biomass (fresh weight) and abundance of DNA reads. +: RT or CA increased earthworm biomass or reads; −: RT or CA decreased earthworm biomass or reads compared to tillage (CT); 0 no difference between the two tillage treatments. The "Agreements" is the number of identical outcomes of comparing the two tillage treatments between the two assessment methods.

		Aulum				Jerslev			
		Oct. 2017		June 2018		Oct. 2017		June 2018	
		FW	DNA	FW	DNA	FW	DNA	FW	DNA
Lumbricus herculeus	An	+	−	0	–	+	0	(−)	(−)
Dendrobaena octaedra	Ep	+	+	Abs.	+	Abs.	–	Abs.	–
Lumbricus rubellus	Ep	Abs.	0	Abs.	+	Abs.	–	Abs.	(−)
Dendrodrilus rubidus	Ep	0	+	Abs.	+	Abs.	Abs.	Abs.	Abs.[1]
Aporrectodea longa	EnAn	Abs.	−	Abs.	+	0	0	0	+
Aporrectodea rosea	En	(−)	−	–	0	0	0	0	–
Aporrectodea tuberculata	En	+	+	0	0	+	–	(+)	–
Aporrectodea caliginosa	En	+	−	Abs.	+	Abs.	–	Abs.	(−)
Allolobophora chlorotica	En	Abs.	Abs.	Abs.	Abs.	0	0	0	+
Agreements		2 of 6		1 of 3		3 of 5		1 of 5	

[1] Very few reads

Table 13. Species lists obtained by metabarconding and by morphological identification from Aulum.

2017		2018	
Metabarcoding	Morphospecies	Metabarcoding	Morphospecies
Ceratophysella denticulata	Arrhopalites caecus	Anurophorus laricis	Arrhopalites caecus
Ceratophysella sp.	**Ceratophysella denticulata**	**Ceratophysella denticulata**	Brachystomella parvula
Deutonura monticola	**Folsomia fimetaria**	**Ceratophysella sp.**	**Ceratophysella denticulata**
Entomobrya atrocincta	**Folsomia quadrioculata**	Deutonura monticola	Cryptopygus thermophilus
Entomobrya marginata	Friesea sp.	Entomobryidae sp.	**Folsomia fimetaria**
Entomobrya unifasciata	**Isotomidae sp.**	Folsomia candida	**Folsomia quadrioculata**
Folsomia candida	**Isotomiella minor**	Folsomia elongata	Friesea sp.
Folsomia elongata	**Isotomurus palustris**	**Folsomia fimetaria**	Isotomidae
Folsomia fimetaria	Lepidocyrtus sp.	**Folsomia quadrioculata**	**Isotomurus palustris**
Folsomia nivalis	**Megalothorax minimus**	Hypogastruridae sp.	**Lepidocyrtus sp.**
Folsomia quadrioculata	Micranurida pygmaea	Isotoma viridis s l	**Megalothorax minimus**
Isotomidae sp.	Mucrosomia garretti	Isotomidae sp.	**Micranurida pygmaea**
Isotomiella minor	Onychiurinae sp.	Isotomiella minor	Mucrosomia garretti
Isotomurus maculatus	**Parisotoma notabilis**	Isotomurus cassagnaui	**Onychiurinae**
Isotomurus palustris	**Sminthurinus elegans**	**Isotomurus maculatus**	**Parisotoma notabilis**
Megalothorax willemi	Stenaphorura quadrispina	**Isotomurus palustris**	**Sminthurinus elegans**
Parisotoma notabilis	Symphypleona sp.	Katiannidae sp.	Stenaphorura quadrispina
Poduromorpha sp.	Tullbergiinae sp.	**Lepidocyrtus cyaneus**	Symphypleona
Protaphorura sp.		**Megalothorax willemi**	Tullbergiinae
Seira delamarei		**Micranurida sp.**	Willemia sp.
Sminthurinus elegans		**Onychiuridae sp.**	
		Orchesella villosa	
		Parisotoma notabilis	
		Parisotoma sp.	
		Protaphorura sp.	
		Seira delamarei	
		Sminthurididae sp.	
		Sminthurinus elegans	
		Sminthurus viridis	
21 species	18 species 9 in common	29 species	20 species 10 in common

Table 14. Species lists obtained by metabarconding and by morphological identification from Jerslev.

2017		2018	
Morphospecies	Metabarcoding	Morphospecies	Metabarcoding
Ceratophysella denticulata	Brachystomella parvula	Bourletiellidae sp.	**Ceratophysella denticulata**
Ceratophysella sp.	**Ceratophysella denticulata**	**Ceratophysella denticulata**	**Entomobrya multifasciata**
Entomobrya multifasciata	**Entomobrya multifasciata**	Entomobrya comparata	**Folsomia fimetaria**
Entomobrya unifasciata	**Folsomia fimetaria**	Entomobrya ligata	**Folsomia quadrioculata**
Entomobryidae sp.	**Folsomia quadrioculata**	**Entomobrya multifasciata**	Folsomides parvulus
Entomobryomorpha sp.	Folsomia spinosa	**Folsomia candida**	Friesea sp.
Folsomia candida	Folsomides parvulus	**Folsomia quadrioculata**	**Isotoma anglicana**
Folsomia fimetaria	**Heteromurus nitidus**	Hypogastrura burkilli	**Isotomurus palustris**
Folsomia quadrioculata	**Isotoma anglicana**	Hypogastruridae sp.	**Lepidocyrtus sp.**
Heteromurus major	Isotomidae sp.	**Isotoma viridis s.l.**	Micranurida pygmaea
Hypogastruridae sp.	**Isotomiella minor**	Isotomidae sp.	**Onychiurinae**
Isotoma viridis	**Isotomurus palustris**	**Isotomurus sp.**	**Parisotoma notabilis**
Isotomidae sp.	**Lepidocyrtus sp.**	**Lepidocyrtus cyaneus**	Pseudosinella alba
Isotomiella minor	**Megalothorax minimus**	Lepidocyrtus lanuginosus	Sminthurinus elegans
Isotomurus maculatus	Micranurida pygmaea	Lepidocyrtus lignorum	Symphypleona
Lepidocyrtus lignorum	Onychiurinae sp.	Lepidocyrtus sp.	**Tullbergiinae**
Lepidocyrtus sp.	Onychiurus cebbenarius	**Onychiuridae sp.**	Willemia sp.
Megalothorax sp.	**Orchesella sp.**	Orchesella villosa	
Megalothorax willemi	**Parisotoma notabilis**	**Parisotoma notabilis**	
Orchesella villosa	Pseudosinella alba	Parisotoma sp.	
Parisotoma notabilis	Pseudosinella sp.	Protaphorura sp.	
Protaphorura sp.	Sminthuridae sp.	Tomocerinae sp.	
Sminthurinus elegans	Sminthurididae sp.	**Tullbergiidae sp.**	
Tomocerinae sp.	**Sminthurinus elegans**		
Tomocerus sp.	Stenaphorura quadrispina		
	Symphypleona sp.		
	Tullbergiinae sp.		
	Willemia sp.		
25 species	28 species	23 species	17 species
	13 in common		10 in common

7 Figures

Legends

Fig. 1. Plot layout at the two study locations Aulum (A) and Jerslev (B).

Fig. 2. Mean earthworm population abundances and biomass per m^2 at the two study locations. Vertical bars are standard error of the mean. Small letters indicate significant differences in measns between the farming systems, P<5%. *: Mean is significantly different from zero.

Fig. 3. Rarefaction curves of the 24 microarthropod samples from each of the two locations in October 2017. The shaded band is the 95% confidence around the estimated species number per sample set.

Fig. 4. PCA of the log transformed earthworm fresh mass showing the first (PC1) and second principal component scores (PC2) representing 44% of the variability.

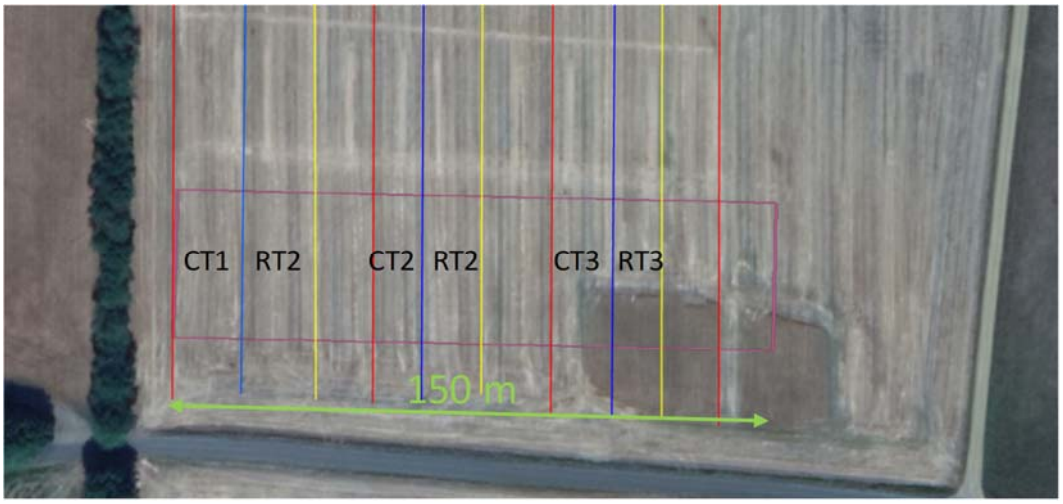
Fig. 5. PCA of the log transformed earthworm read abundances showing the first (PC1) and second principal component scores (PC2) representing 43% of the variability.

Fig. 6. PCA of the log transformed relative read abundances of the most abundant collembolan and Acari OTUs from 2017 showing the first (PC1) and second principal component scores (PC2) representing 20% of the variability.

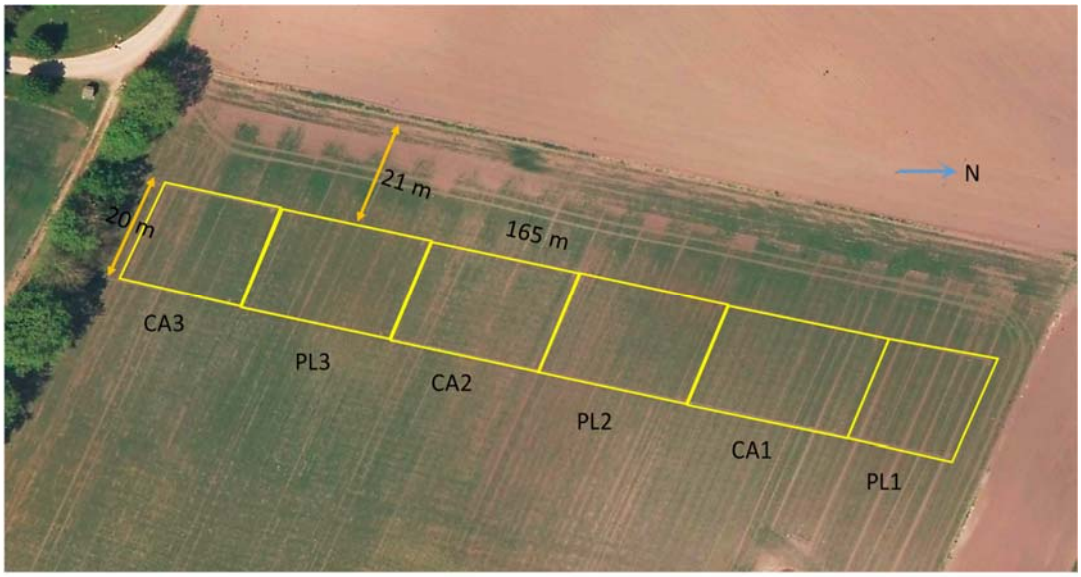
Fig. 7. PCA of the log transformed relative read abundances of collembolans in 2017 showing the first (PC1) and second principal component scores (PC2) representing 18% of the variability.

Fig. 8. PCA of the log transformed relative read abundances of collembolans in 2018 showing the first (PC1) and second principal component scores (PC2) representing 20% of the variability.

Fig. 1.


CT1
RT2
CT2
RT2
CT3
RT3
150 m

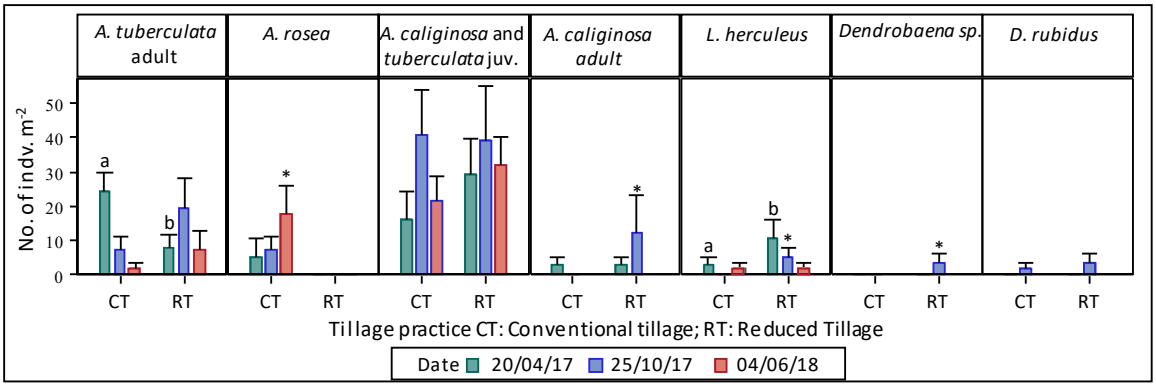
A

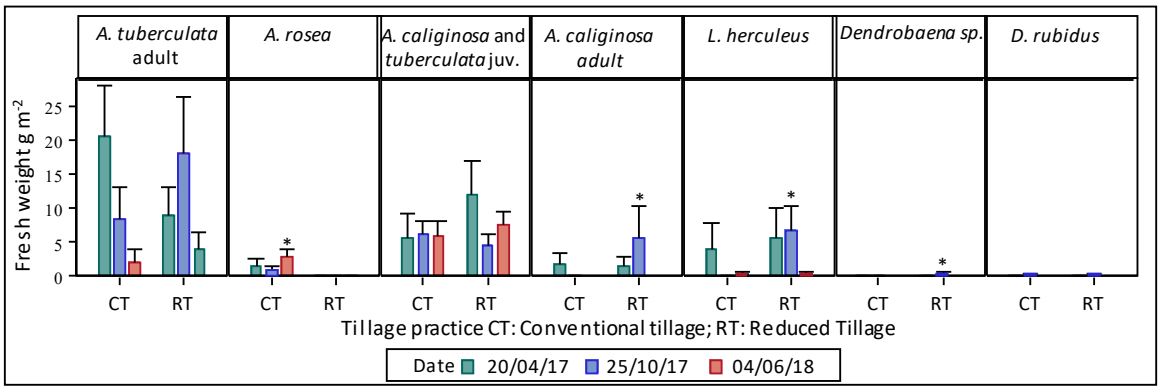

21 m
20 m
165 m
N
CA3
PL3
CA2
PL2
CA1
PL1

B

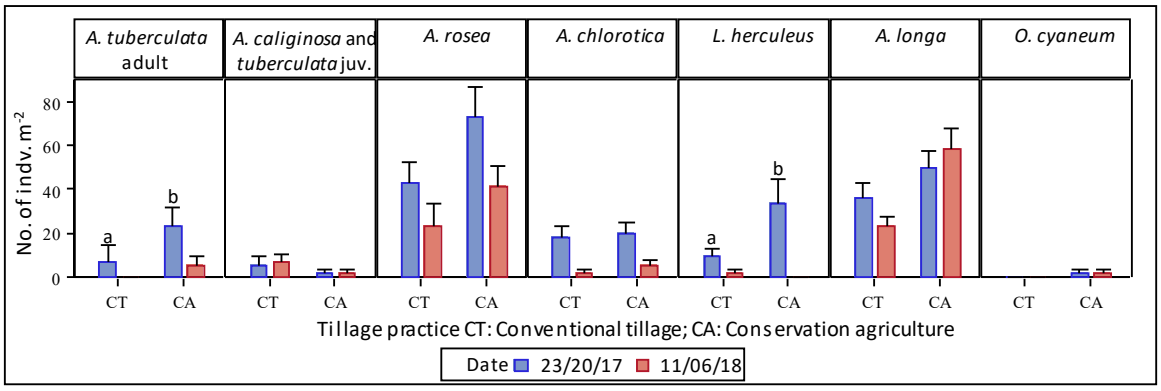
Fig. 2.

Aulum





Jerslev



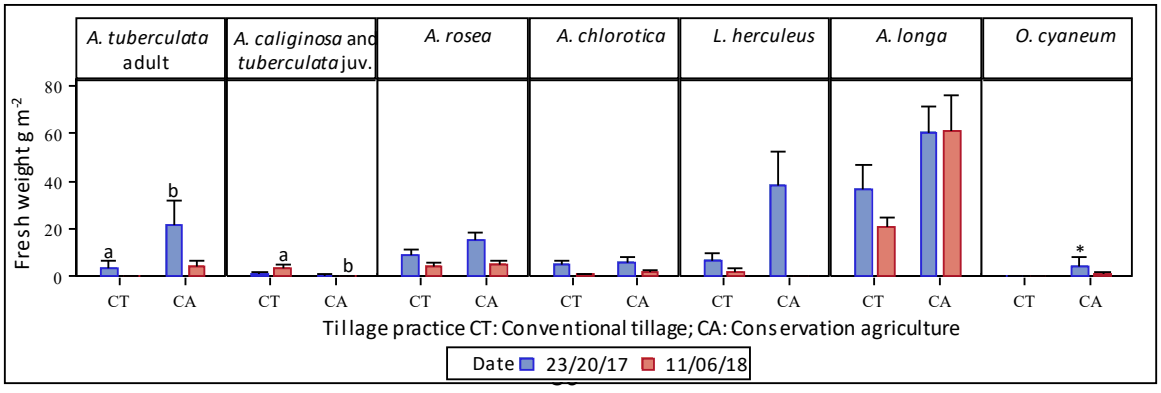


Fig. 3.

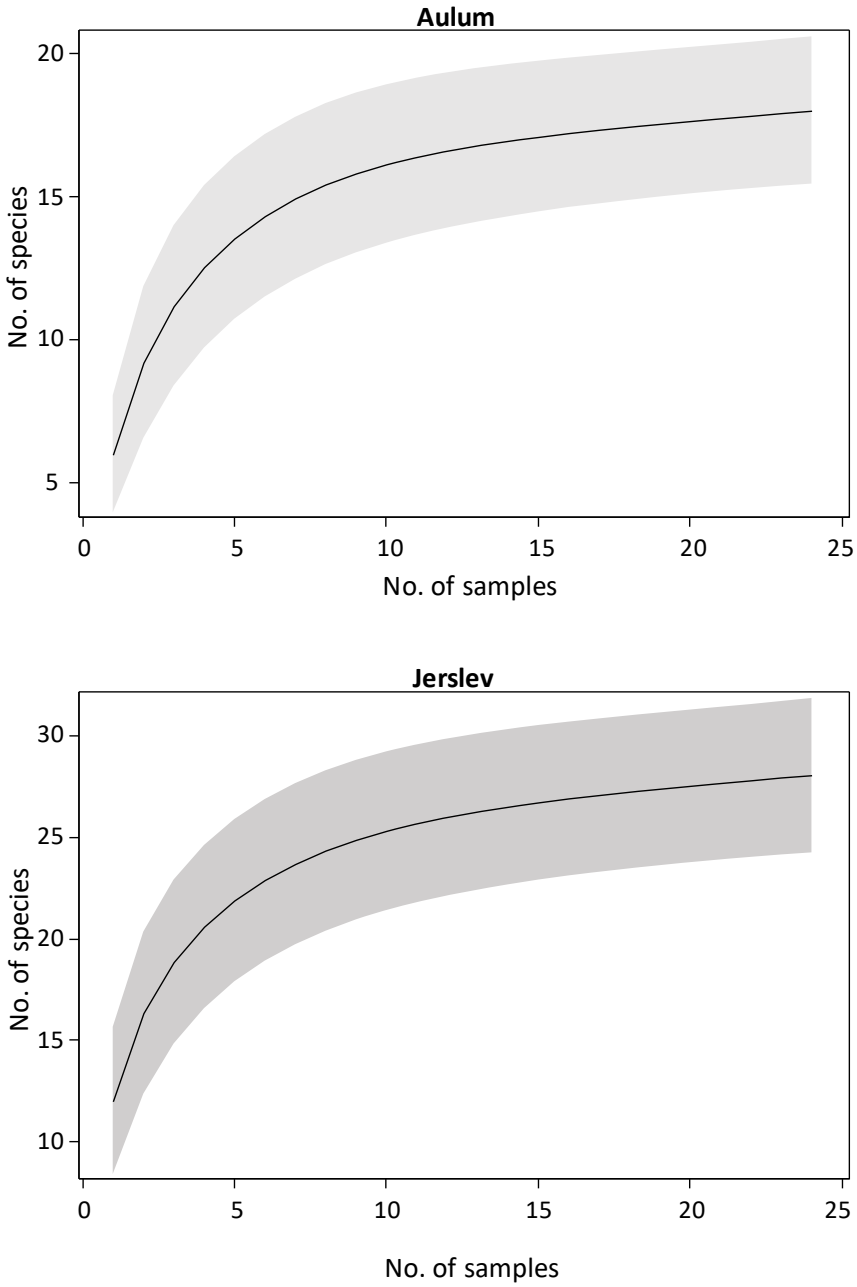

Aulum
No. of species
No. of samples
20
15
10
5
0
5
10
15
20
25
Jerslev
No. of species
No. of samples
30
25
20
15
10
0
5
10
15
20
25

Fig. 4.

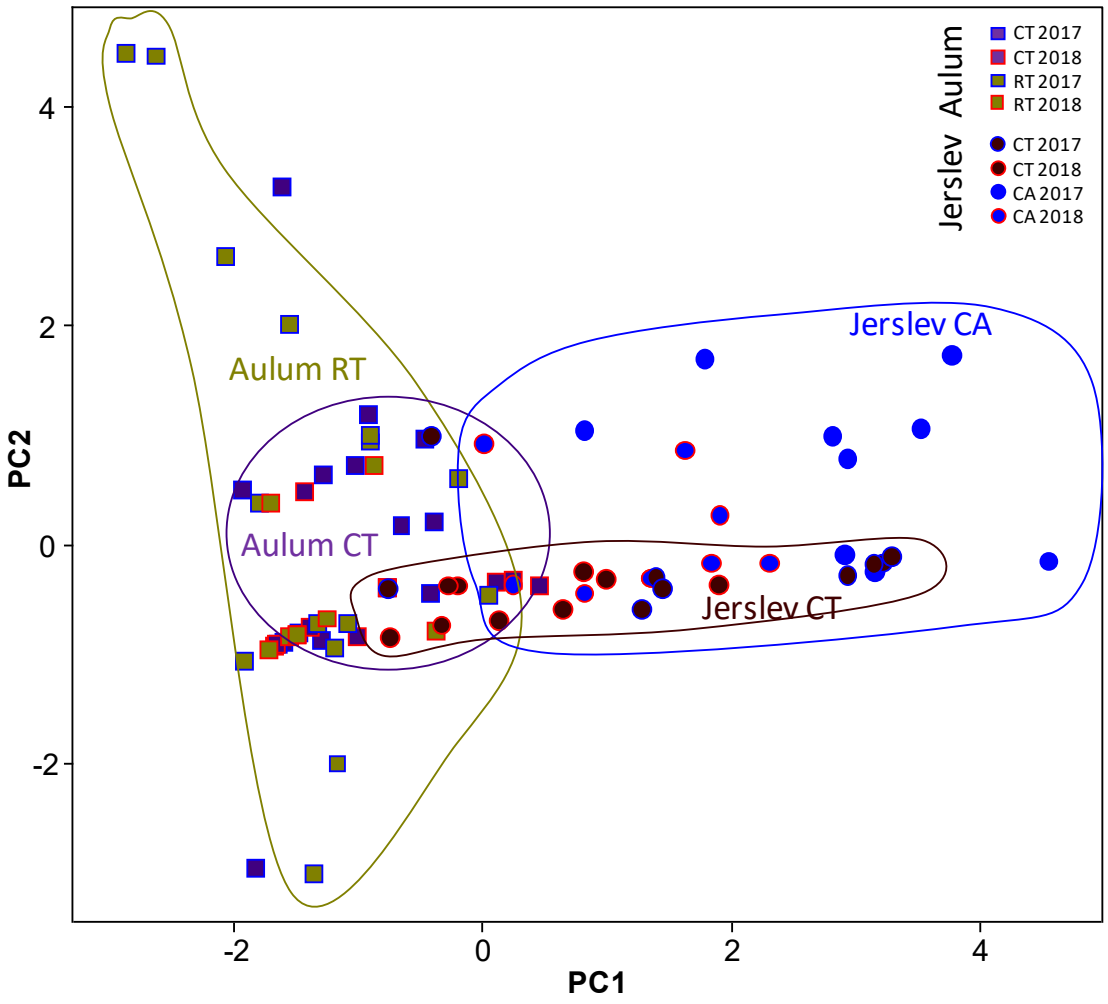
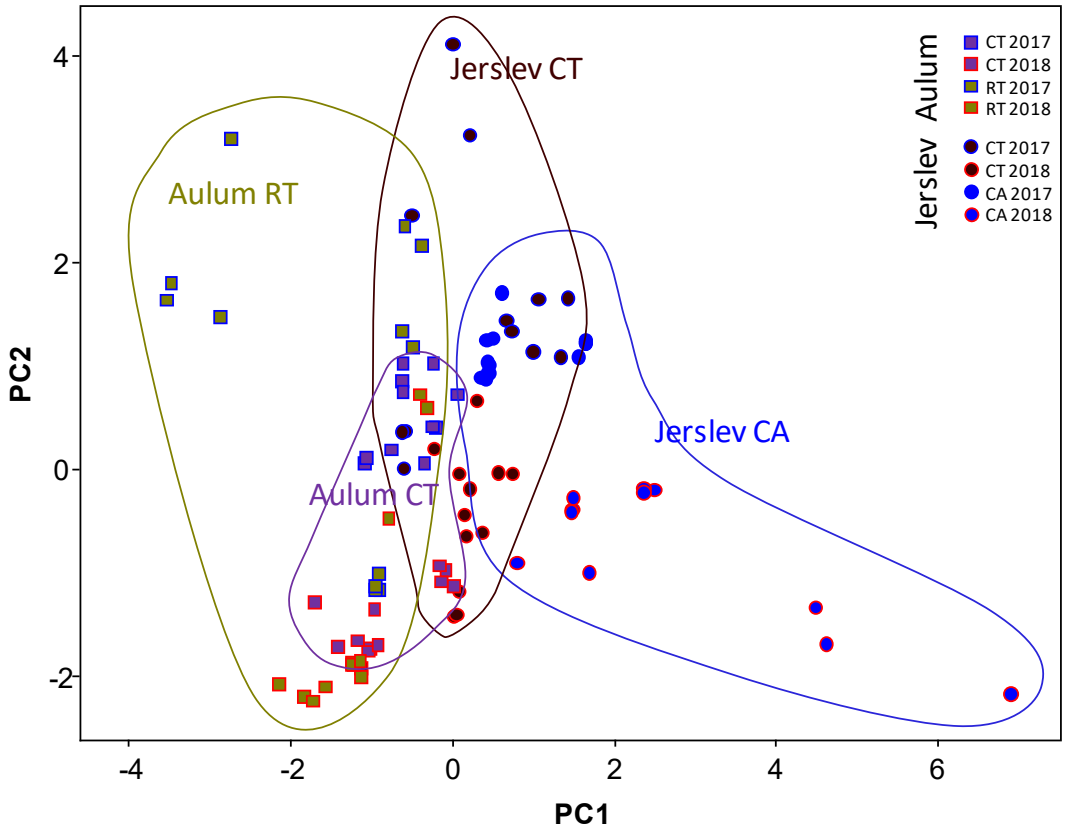

Jerslev Aulum
CT 2017
CT 2018
RT 2017
RT 2018
CT 2017
CT 2018
CA 2017
CA 2018
Aulum RT
Aulum CT
Jerslev CA
Jerslev CT
PC1
PC2
4
2
0
-2
-2
0
2
4

Fig. 5.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Aulum RT
Aulum CT
Jerslev CA
Jerslev Aulum
CT 2017
CT 2018
RT 2017
RT 2018
CT 2017
CT 2018
CA 2017
CA 2018
PC2
PC1
4
2
0
-2
-4
-2
0
2
4
6

Fig. 6.

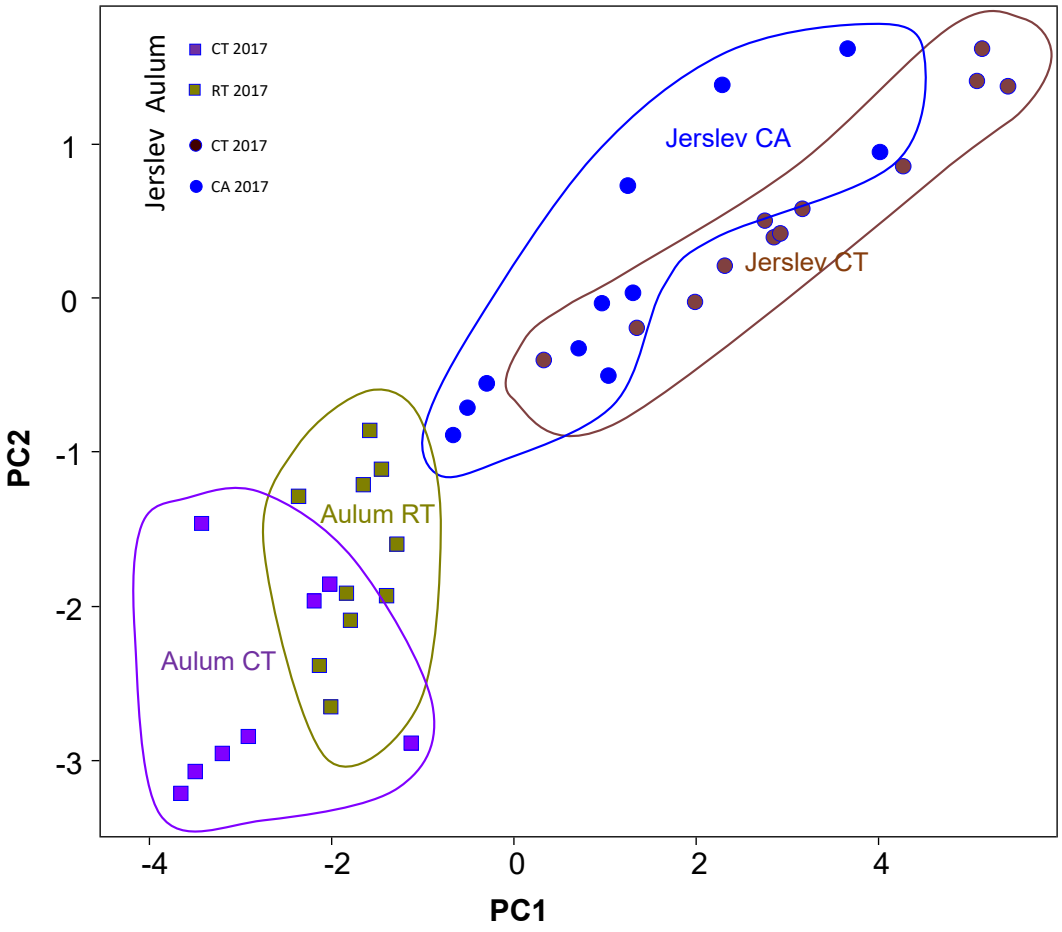

Jerslev Aulum
CT 2017
RT 2017
CT 2017
CA 2017
Jerslev CA
Jerslev CT
Aulum RT
Aulum CT
PC2
PC1
1
0
-1
-2
-3
-4
-2
0
2
4

Fig. 7.

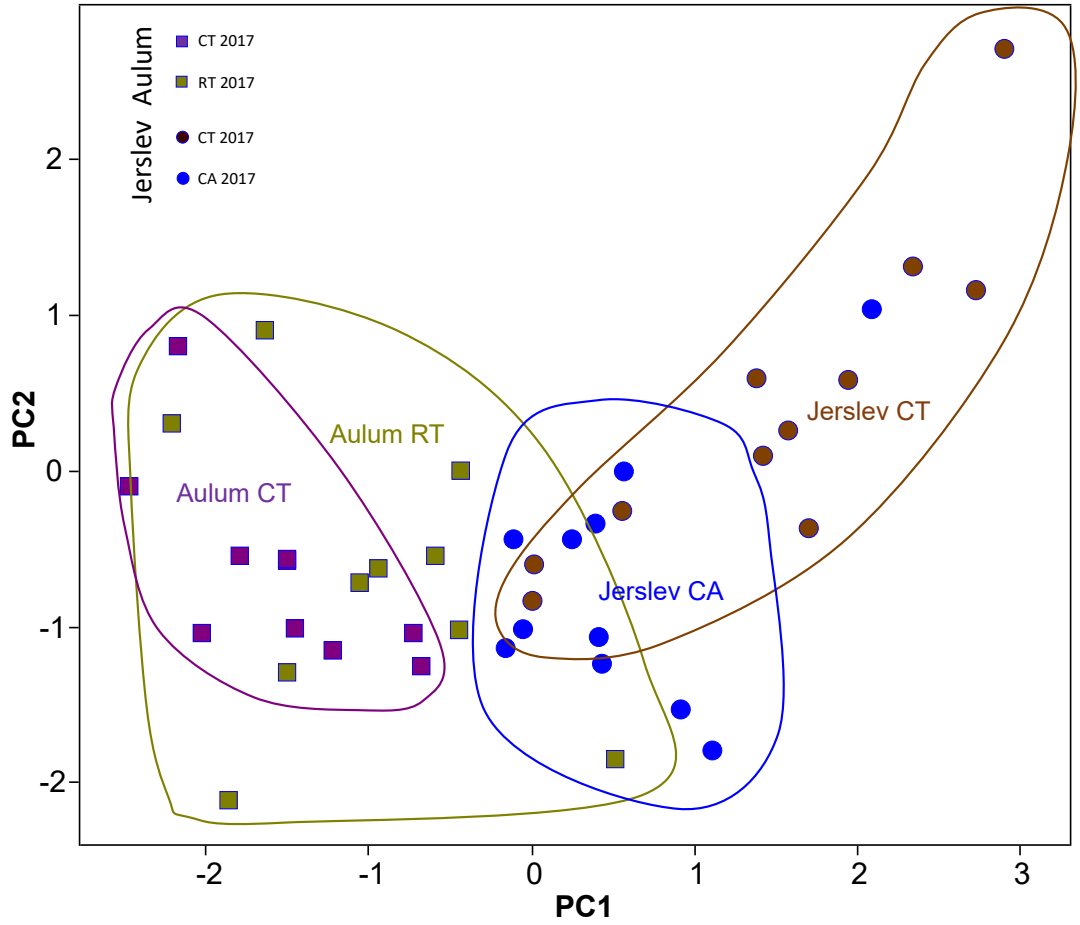
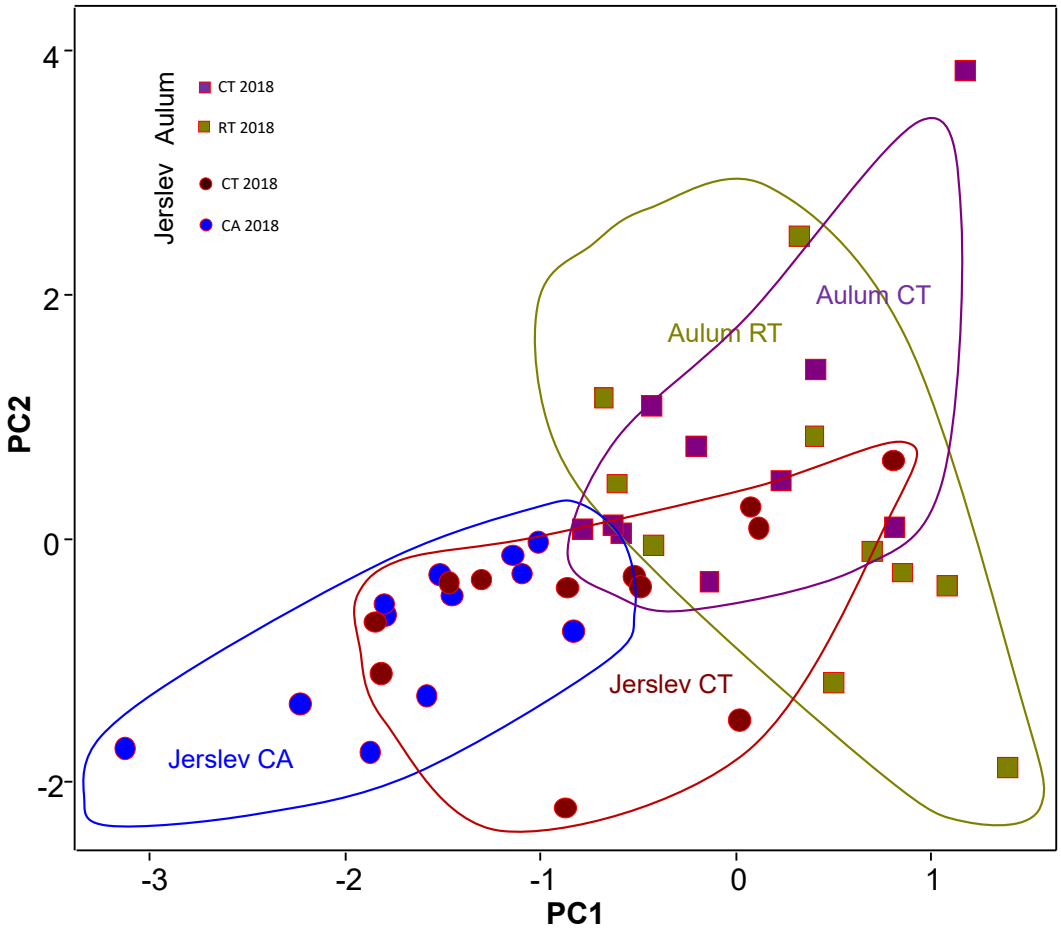

Jerslev Aulum
CT 2017
RT 2017
CT 2017
CA 2017
Aulum RT
Aulum CT
Jerslev CT
Jerslev CA
PC2
PC1
2
1
0
-1
-2
-2
-1
0
1
2
3

Fig. 8.


Jerslev Aulum
CT 2018
RT 2018
CT 2018
CA 2018
Aulum CT
Aulum RT
Jerslev CT
Jerslev CA
PC2
PC1
4
2
0
-2
-3
-2
-1
0
1