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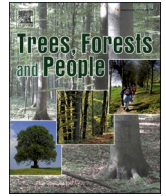
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## Cadmium accumulation in organisms from a spruce plantation amended with wood ash - an environmental risk?

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### ABSTRACT

Recycling of wood ash from power plants to forest plantations returns plant nutrients lost at harvest. However, wood ash contains a considerable amount of cadmium (Cd) that may accumulate in the forest food web, and eventually threaten environment and human health. We examined the short-term (6–24 months) uptake of Cd in a range of organisms from different trophic levels in the forest food web. We amended twelve field plots in a Norway spruce plantation with wood ash in different concentrations (0, 3, 4.5 and 6 t ha<sup>-1</sup>), and subsequently measured Cd concentration in three vascular plant species, three metazoan groups, a lichen, and fruiting bodies of four ectomycorrhizal fungal species. Cd content varied significantly between the major taxonomic groups; metazoans contained most and vascular plants least, but also within the groups, most pronouncedly for ectomycorrhizal basidiomycetes. In vascular plants, metazoans as well as fungi, we found some examples that Cd content correlated significantly with wood ash amendment, however most organisms were unaffected, and no group of organisms contained more Cd than allowed for comparable food items. We therefore conclude that with the wood ash dosages used here, the environmental risk of Cd accumulation is slight, at least in the forest system that we examined.

### Introduction

Wood is increasingly used as an alternative to fossil fuels, but removal of wood from plantations results in export of plant nutrients. Most nutrients (except nitrogen) are retained in the ash after combustion and recycling of wood ash to plantations is therefore desirable (Pitman 2006; Huotari et al., 2015). However, wood ash also contains harmful trace elements (Pitman 2006), and therefore safety-limits regulate the amount of wood ash to be recycled. In Denmark, the maximum amount of wood ash to be applied in plantations is 3 t ha<sup>-1</sup> per 25 years, and other countries have recommendations that are very similar (Stupak et al., 2007). To obtain a circular economy of nutrients, it might be desirable to increase these safety limits, but this requires knowledge on

potentially harmful side-effects. We focus on the trace element cadmium (Cd), often considered the primary problem in wood ash. Cd is highly toxic and may accumulate in organisms and thus jeopardize ecosystem functioning and human health (Godt et al., 2006; Jarup and Akesson 2009). Hence, it is a concern whether the amendment will result in a pulse of increased Cd concentrations in the food web (Mortensen et al., 2018).

Cd is actively taken up by organisms due to chemical similarities with the essential micro-nutrient Zn (Clemens 2006), therefore it has high potential for bio-accumulation. Increased Cd concentration in soil usually leads to increased uptake in soil organisms. However, wood ash is a complex substance, and it may also contain components that impede Cd uptake in soil organisms (Johansen et al., 2021). Firstly, wood ash is

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**Table 1**

Average pH and heavy metal concentration (mean  $\pm$  SD) in soils of plots treated with 0, 3, 4.5 or 6 t ha<sup>-1</sup> wood ash. The heavy metals we focus on in this study Cd is highlighted in bold. Note that both pH and Zn concentration may affect Cd accumulation in organisms.

	pH	Cd (mg kg <sup>-1</sup> )	Cr (mg kg <sup>-1</sup> )	Cu (mg kg <sup>-1</sup> )	Ni (mg kg <sup>-1</sup> )	Pb (mg kg <sup>-1</sup> )	Zn (mg kg <sup>-1</sup> )
0 t ha <sup>-1</sup>	3.3 $\pm$ 0.2	<b>0.29 <math>\pm</math> 0.19</b>	2.2 $\pm$ 0.7	5.0 $\pm$ 2.2	2.1 $\pm$ 1.7	24 $\pm$ 6.5	14.3 $\pm$ 5.0
3 t ha <sup>-1</sup>	3.9 $\pm$ 0.2	<b>0.30 <math>\pm</math> 0.08</b>	2.6 $\pm$ 0.6	4.6 $\pm$ 2.3	1.7 $\pm$ 0.5	26 $\pm$ 6.6	18.7 $\pm$ 8.0
4.5 t ha <sup>-1</sup>	4.0 $\pm$ 0.4	<b>0.33 <math>\pm</math> 0.08</b>	3.1 $\pm$ 1.0	5.6 $\pm$ 1.6	2.5 $\pm$ 1.0	30 $\pm$ 5.0	19.0 $\pm$ 4.0
6 t ha <sup>-1</sup>	4.1 $\pm$ 0.2	<b>0.37 <math>\pm</math> 0.03</b>	2.9 $\pm$ 0.4	5.1 $\pm$ 1.3	2.1 $\pm$ 0.2	27 $\pm$ 3.5	20.2 $\pm$ 6.0

highly alkaline and increases the soil pH, which decreases the bio-availability of Cd (Bradl 2004; Johansen et al., 2019; Kindtler et al., 2019). On the other hand, the combustion process may alter the chemical composition and produce more readily available Cd compounds. Lastly, wood ash also contains a substantial amount of Zn, which decreases Cd accumulation in plants (Oliver et al., 1994). Thus, it is a challenge to predict Cd uptake following wood ash application and in situ studies under realistic management regimes and ash dosages are most wanted.

Here, we therefore aimed to describe the relation between wood ash amendment and Cd content in organisms from different positions in the food web. In addition, we aimed to evaluate the risk of any increases in Cd content of the organisms. To investigate this, we used a field experiment in a Norway spruce plantation and treated the soil with four levels of wood ash (0, 3, 4.5 and 6 t ha<sup>-1</sup>). We collected organisms that have different positions in the food web, measured their Cd content, and correlated it with the wood ash amendment.

## Methods

### Field site description

Our field site is a *Picea abies* L. (Norway spruce) plantation (Gedhus plantation, Karup, Denmark, N 56° 16.633', E 9° 05.200'). The vegetation consists mostly of bryophytes with only few and scattered vascular plants, mainly *Deschampsia flexuosa* (L.) Trin and *Vaccinium vitis-idaea* L. The soil is a nutrient poor podzol with 10 cm organic layer and very acidic (pH=3.2) podzol. Maresca et al. (2018) provide a detailed description of the soil on this field site. In April 2014, we established 12 plots (20  $\times$  25 m<sup>2</sup>) and treated them with wood ash in levels of 0, 3, 4.5 and 6 t ha<sup>-1</sup>; with each amendment level replicated three times in a

**Table 2**

Average Cd content in plants, metazoans and fungi collected in field plots amended with four different concentrations of wood ash, 0 t ha<sup>-1</sup>, 3 t ha<sup>-1</sup> (the currently allowed dose), 4.5 t ha<sup>-1</sup>, and 6 t ha<sup>-1</sup>. The three major taxonomic groups (plants, metazoans and fungi) contained significantly different amounts of Cd ( $p < 0.001$ , one way ANOVA). The table also shows p-values for linear correlation between organism Cd and wood ash amendment. In only 3 of 11 organisms (p-values in bold); we found a significant correlation between amended wood ash and Cd content (correlations shown in Fig. 2).

		Average Cd content ( $\mu$ g kg <sup>-1</sup> dry weight)	R <sup>2</sup>	p
Plants	<i>Deschampsia flexuosa</i>	79.6	0.5	<b>0.01</b>
	<i>Vaccinium vitis-idea</i> , old leaves (2015)*	27.8	0.04	0.53
	<i>Vaccinium vitis-idea</i> , new leaves (2016)	35.8	0.005	0.87
	<i>Picea abies</i> 2016	18	0.0001	0.98
Metazoans	<i>Picea abies</i> 2013*	6.7	0.02	0.68
	Gamasid mites	1704	0.03	0.65
	<i>Nothrus</i> sp. mites	2193	0.003	0.89
Fungi	<i>Dendrobaena octaedra</i>	4486	0.52	<b>0.008</b>
	<i>Cladonia</i> sp.	125.8	0.42	<b>0.03</b>
	<i>Lactarius</i> sp.	758.6	0.05	0.59
	<i>Russula</i> sp.	991.6	0.0005	0.96
	<i>Paxillus involutus</i>	71.1	0.14	0.23
	<i>Thelephora terrestris</i>	126.4	0.42	0.34

\* note that the older leaves were also sampled in from plant material in 2016.

randomized block design. A buffer zone of 3 m separated each plot (Hansen et al., 2018). We then evenly distributed the wood ash on the forest floor using a hand shovel.

### Wood ash

We used wood ash from the incineration of *P. abies* wood chips at Brande Heating Plant (Brande, Denmark, N 55°56'39.757" E 9°7'20.110"). The wood ash was a mixture of bottom- and fly ash and had not been exposed to any stabilization treatment before addition in compliance with Danish legislation. The wood ash mainly consists of alkaline calcium salts and therefore has a high pH (13.2) and it contains 3.99 mg Cd kg<sup>-1</sup>. Further chemical details are provided by Maresca et al. (2017).

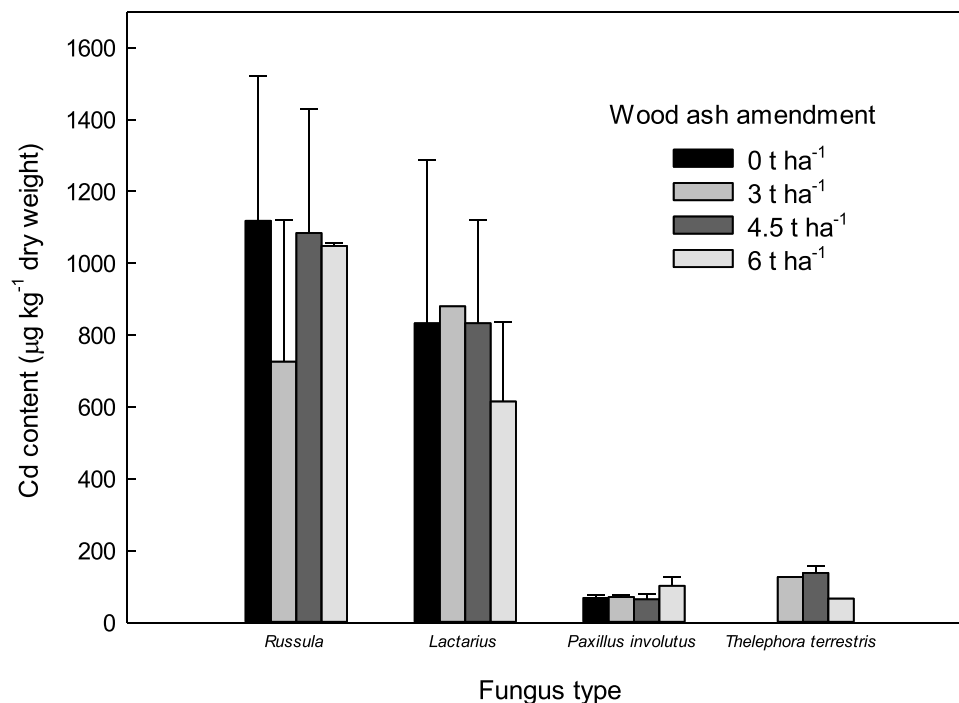
### Soil measurements

We sampled soil from the top 0–10 layer of the field plots in 2017. Soil pH was measured on soil slurries (soil to water ratio was 1:5) with a glass electrode (PHM240 pH/ION METER, MeterLab). The remaining soil sample was dried and homogenised. The aliquots of 1 g of soil sample were digested in 40 mL 32.5% HNO<sub>3</sub> using the "plant material" program on a CEM MARS microwave (CEM, North Carolina, USA) and concentration of elements were measured by Atomic Absorption spectrometry (Perkin Elmer PinAAcle 900 T) with the graphite furnace technique.

### Organisms and cadmium measurements

We sampled fruiting bodies of the four ectomycorrhizal fungi *Russula* spp., *Lactarius* spp., *Thelephora terrestris*, *Paxillus involutus* and the lichen *Cladonia* spp. in October 2014. Aboveground material of *D. flexuosa*, *P. abies* and *V. vitis-idaea* as well as soil cores for extraction of metazoans were sampled in August 2016. Sampling time-points were chosen to reflect expected response time of the different organisms as to obtain maximum effect.

For *P. abies*, we sampled both fresh needles produced in 2016 as well as older needles from 2013 (i.e. produced before the wood ash was spread). Similarly for *V. vitis-idaea*, we sampled freshly produced leaves from 2016 as well as last year's leaves from 2015. We sampled five soil cores from each plot (diameter: 6 cm), to a depth of 0–5 cm and extracted mites using the Tullgren funnel method (Macfadyen 1953). From each of the extracted mite samples, we picked at least 100 individual specimens of the carnivorous superfamily Gamasida and the detritivorous genus *Nothrus* (Capinera 2008). The only earthworm species present at the site, *Dendrobaena octaedra*, was obtained by hand sorting from 10 cm diameter soil cores. Prior to analysis, the earthworm digestive tracts were evacuated. We washed all organisms with ddH<sub>2</sub>O and dried them for 24 h at 60 °C. Plant and fungal material was crushed (Pulverisette 23 mini-mill, FRITSCH, Idar-Oberstein, Germany). All samples were added to MARSXpress Teflon tubes (CEM, Matthews, North Carolina, USA), supplied with 32.5% HNO<sub>3</sub>, and digested on a turntable in a MARS 6 (CEM, Matthews, North Carolina, USA). After further dilution to 6.5 % HNO<sub>3</sub>, Cd was measured on a PinAAcle 900T atomic absorption spectrometer (PerkinElmer, Waltham, Massachusetts, USA).



**Fig. 1.** Cd content in fruit bodies of four types of ectomycorrhizal fungi collected in field plots amended with wood ash in dosages of 0 t ha<sup>-1</sup>, 3 t ha<sup>-1</sup> (the recommended safe dose), 4.5 t ha<sup>-1</sup>, and 6 t ha<sup>-1</sup>. Wood ash amendment had no significant effect on fruit-body concentration of Cd ( $p = 0.96$ ), neither did we find an interaction between wood ash amendment and fungi ( $p = 0.59$ ), whereas Cd content differed significantly between fungi ( $p < 0.0001$ , two way ANOVA, data log transformed to obtain variance homogeneity). A Tukey HSD test showed that the fruit-bodies fell in two groups; 1: *Lactarius* and *Russula*, and 2: *Thelephora terrestris* and *Paxillus involutus* ( $p < 0.003$ ). Error-bars show 1 SE.

#### Data analyses

For each of the 12 plots in the randomised plot design experiment, we obtained one value. Thus, for each of the four treatments, we had three replicates for further statistical analyses. We used ANOVA models to analyse the effect of wood ash amendment and to compare taxonomic groups. Block was included as a factor in all ANOVA analyses, but subsequently removed from the final model as it did not explain variation in the data. Additionally, we performed linear regression analyses on each taxonomic group with wood ash amendment as the independent variable and Cd concentration as dependant variable. ANOVA models were performed in SAS Enterprise Guide 7.1 and linear models were performed in SigmaPlot 13.0.

#### Results and discussion

##### *Different types of organisms accumulated very different amounts of Cd*

Data for soil pH and heavy metal concentrations after ash addition are presented in Table 1. We found an increased pH and moderate higher heavy metal concentrations. We recorded a significant difference in Cd content between the three major taxonomic groups, i.e. plants, fungi and metazoans ( $p < 0.001$ , one way ANOVA; Table 2). Trace metal properties, soil chemistry (e.g. soil pH and Zn concentration) will, in combination with organism foraging strategy and physiology, influence trace metal accumulation in particular organisms. However, in particular, one overall difference between our target groups is their protein content. Proteins are key targets of trace elements (Tamás et al., 2014). Metazoans generally contain more protein than fungi that contain more protein than vascular plants.

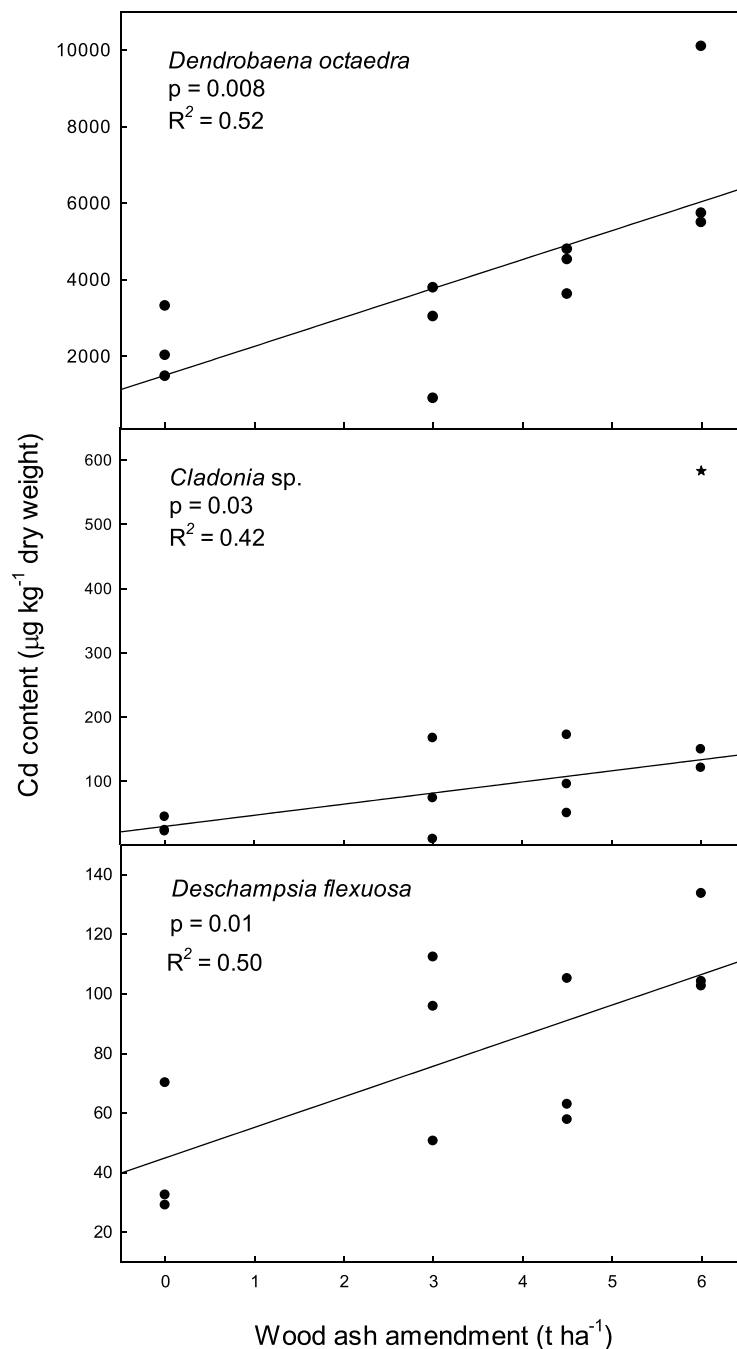
Cd content in fruiting bodies of ectomycorrhizal fungi differed significantly (Fig. 1); *Russula* and *Lactarius* contained much more than *Thelephora* and *Paxillus*. Similar differences have not previously been reported in the literature (Lodenius et al., 2002; Moilanen et al., 2006;

Omil et al., 2007; Krpata et al., 2009; Gil-Martínez et al., 2020), however, fungal hyphae immobilize Cd; the more mycelia produced the more Cd is taken up (Galli et al., 1994). *Russula* and *Lactarius* are mainly contact distance exploration types with little extra-radical mycelia production, while *Thelephora* and *Paxillus* are distance exploration types (Agerer 2001). Hence, patches with different amount of mycelia that absorb Cd to different extent and/or distance from potential uptake of Cd to fruiting body may affect Cd translocation. Further experiments should map the Cd content of the different ectomycorrhizal compartments using root tip level analysis and mycelial in-growth systems (Kjøller 2006).

The earthworm *Dendrobaena octaedra* contained considerably more Cd than the mites (Table 1). Cd content in soil metazoans differs markedly (Janssen et al., 1993; Janssen and Hogervorst 1993), and often independently of Cd exposure (Heikens et al., 2001), because species specific physiology, rather than exposure level determines Cd accumulation. Ability to excrete Cd is an important mechanism (Janssen and Hogervorst 1993; Mortensen et al., 2018), and earthworms do not excrete, but rather sequester Cd in the gut lining (Andersen and Laursen 1982). Here Cd is detoxified in metallothioneins that facilitate accumulation without detrimental effects (Klaassen et al., 2009). Although the two groups of mites, Gamasida and the genus *Nothrus*, represent different trophic levels, their Cd contents were not significantly different. Likewise, Roberts and Johnson (1978) and Van Straalen and Van Wensem (1986) reported that trophic position does not correlate with metal accumulation in soil invertebrates.

##### *Cd content only varies with wood ash amendment for some organisms*

Only for three of the organisms, Cd content increased significantly with wood ash amendment, the grass *D. flexuosa*, the lichen *Cladonia*, and the earthworm *Dendrobaena octaedra* (Fig. 2). *D. flexuosa* most often forms arbuscular mycorrhiza (Vosatka and Dodd 1998) which acts as a filter for their associated plant and relieves trace element stress



**Fig. 2.** Linear correlations between wood ash amendment and Cd content in organisms collected in field plots amended with four levels of wood ash, 0 t ha<sup>-1</sup>, 3 t ha<sup>-1</sup> (the recommended safe dose), 4.5 t ha<sup>-1</sup>, and 6 t ha<sup>-1</sup>. A-C: The figure only shows relationships for organisms, where the correlation was significant. Note that in *Cladonia*, a possible outlier (marked as a star) was removed prior to analysis to obtain variance homogeneity.

(Hildebrandt et al., 2007). However, in our study site no mycorrhizal colonization was observed (R. Kjølner, personal observation), which may explain its higher Cd-uptake. For *Cladonia*, Osyczka and Rola (2013) and Sueoka et al. (2016) also showed that trace element content correlates with substrate content. For several reasons, earthworms are more likely to accumulate Cd in sites amended with wood ash. Earthworms have a highly permeable cuticle and combined with their rather indiscriminating engulfment of soil particles (including wood ash) (Lanno et al., 2004), this makes them highly exposed. Further, earthworms have a gizzard containing small particles taken up from the environment (Edwards and Bohlen 1996). Here, they will also occasionally take up small solid ash-particles. Finally, as mentioned above, earthworms sequester and detoxify Cd in the gut lining.

#### Does the Cd in the organisms present a risk?

The Cd from the ash may potentially harm e.g., birds feeding on earthworms or other invertebrates. Birds may suffer from significant physiological harm, also at trace metal concentrations insufficient to cause overt toxicity. Particularly, reproductive effects as decreased egg production or hatchability, and increased hatchling mortality (Scheuhammer 1987). Similarly, human mushroom collectors may be affected, as fungi may accumulate trace elements (Gadd 1993). We compared our findings to the official EU list of maximum levels for contaminants in foodstuffs (EU 2006). We notice that values for “Leaf vegetables, fresh herbs, cultivated fungi” and Cephalopods (the phylogenetically closest relatives we can identify to the invertebrates we sampled) are 200 and

1000  $\mu\text{g kg}^{-1}$ , respectively. Thus, the values we recorded for the green plant parts are considerable below these values, which are even for fresh and not dry weight. Both fungal fruit-bodies and earthworms had higher Cd contents, but they fall within the natural range for these organisms (Moilanen et al., 2006; Rozen 2006). The concentrations are in the same order of magnitude as the EU maximum values, but these are also fresh weight values; dry weight of the fungal fruiting-bodies and earthworms are approximately 10 % of the fresh weight. We found one single worm in the 6 t ha<sup>-1</sup> treatment, with a Cd content of 30,000  $\mu\text{g kg}^{-1}$ , corresponding to 3000  $\mu\text{g kg}^{-1}$  fresh weight. With this exception, all organisms contained Cd below the recommended maximum values.

## Conclusion

We conclude that wood ash amendment in plantations poses a very low risk in regard to Cd bioaccumulation. Three organisms had a significantly higher Cd content with increasing wood ash doses, whereas eight had not. However, Cd concentrations were far below the EU recommended limits for food. Cd content varied significantly between organisms independently of wood ash dosage.

## CRedit authorship contribution statement

**Flemming Ekelund:** Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. **Carla Cruz-Paredes:** Christian Hviid Friis Jørgensen: Data curation, Writing – review & editing. **Mette Vestergård:** Data curation, Supervision, Writing – review & editing. **Kathrine Rostgaard Rasmussen:** Louise Hindborg Mortensen: Writing – review & editing. **Nikolaj Lunding Kindtler:** Data curation, Supervision, Writing – review & editing. **Regin Rønn:** Data curation, Writing – review & editing. **Søren Christensen:** Funding acquisition, Writing – review & editing. **Jesper Liengaard Johansen:** Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Rasmus Kjølner:** Data curation, Funding acquisition, Writing – original draft, Writing – review & editing.

## Declaration of competing interest

The authors declare no conflict of interest

## Data availability

Data will be made available on request.

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