Effect of spatial resolution of soil data on predictions of eggshell trace element levels in the Rook Corvus frugilegus

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Although a considerable research effort has gone into studying the dietary pathways of metals to the bodies of laying female birds and their eggs in recent years, no detailed investigations have yet been carried out relating the properties of the biogeochemical environment at large spatial scales to eggshell trace element levels in typical soil-invertebrate feeding birds under natural conditions. We used data from a large-scale nationwide monitoring survey of soil quality in Poland (3724 sampling points from the 43,792 available) to predict levels of five trace elements (copper [Cu], cadmium [Cd], nickel [Ni], zinc [Zn] and lead [Pb]) in Rook Corvus frugilegus eggshells from 42 breeding colonies. Our major aim was to test whether differences exist in the explanatory power of soil data (acidity, content of elements and organic matter, and particle size) used as a correlate of concentrations of eggshell trace elements among four different distances (5, 10, 15 and 20 km) around rookeries. Over all four distances around the rookeries only the concentrations of Cu and Cd in eggshells were positively correlated with those in soil, while eggshell Pb was correlated with the soil Pb level at the two longest distances (15 and 20 km) around the rookeries. The physical properties of soil (primarily the increase in pH) adversely affected eggshell Cd and Pb concentrations. The patterns and factors governing metal bioaccumulation in soil invertebrates and eggshells appear to be coincident, which strongly suggests a general similarity in the biochemical pathways of elements at different levels of the food web. The increasing acidification of arable soil as a result of excessive fertilisation and over-nitrification can enhance the bioavailability of toxic elements to laying females and their eggs.

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1 Introduction

Soil is a critically important component of the earth’s biosphere, playing a key role in ecosystem functioning and the maintenance of local, regional and global environmental quality (Glanz, 1995; Doran, 2002; Gregory et al., 2015). Physicochemical properties such as acidity and levels of organic matter and chemical elements are among the most important features of soil responsible for the functioning of organisms (reviewed in Ardestani et al., 2014). In the soil environment, all these properties showed close additivity and can to different degrees be responsible for the bioavailability and transfer of trace elements, such as toxic metals, from soil to soil invertebrates, and generally further through terrestrial food webs (Heikens et al., 2001; Zhuang et al., 2009; Schipper et al., 2012; Ardestani et al., 2014).

In arable top soil most trace elements are present at higher levels than in the natural bedrock, mostly because of the accumulation of anthropogenic nutrients (e.g. from agriculture and industrial processes) in the soil organic matter (Kabata-Pendias and
Pendas, 2000), Bradham et al. (2006) found soil pH to be inversely related to the bioavailability of toxic elements. Other studies have suggested that fine soil particles are responsible for retaining potentially toxic metals: the clay fraction (<0.002 mm) in particular contained the highest amounts of heavy metals (cadmium [Cd], chromium [Cr], copper [Cu], lead [Pb] and zinc [Zn]) (Yutong et al., 2016).

In a global sense, increasing soil acidity is due to anthropogenic disturbances (Tilman et al., 2001; Guo et al., 2010) and is exacerbated by the continuing reliance on nitrogen and phosphate fertilisers, a practice known to accelerate leaching of basic cations from soils (Ormerod and Rundle, 1998; see McCallum et al., 2015). Altering the biogeochemistry of ecosystems and adversely affects biota, including birds, this can lead to impaired breeding in an acid-stressed environment, principally as a result of calcium deficiency during the period when female birds are producing eggs (Scheuhammer, 1991; Dauwe et al., 2006) or even a reduced bird abundance (Pabian and Bittingham, 2007).

Avian eggs and their shells are good indicators of pollution by trace elements such as Pb. Its chemical properties are similar to those of calcium (Ca), that is, it competes for binding sites and is transported and stored in the same way as Ca (Scheuhammer, 1987; Ruuskanen et al., 2014; Hashmi et al., 2015). It is assumed that female birds can sequester the surplus of certain trace elements, particularly non-essential ones, in the eggshell (Burger, 1994; Dauwe et al., 1999; Mora, 2003; Hashmi et al., 2015). As bird embryos use the eggshell as a source of calcium and other elements for growth (Blom and Lilja, 2004), the presence of some metals at high levels in the shell may adversely affect embryonic development (Ortowski et al., 2016). The concentrations of some trace elements measured in eggshells reflect circulating metals in the blood of the female at the time of egg-laying; in addition, positive correlations have been found between metal levels in the blood and other tissues of parent birds and their eggs (see Ruuskanen et al., 2014). In migratory species, metal levels measured in internal organs (e.g. the liver) rise shortly after arriving at polluted sites (Berglund et al., 2011). This implies strongly that metal levels in eggs reflect recent and local exposure (at the breeding grounds) to pollution of the females that laid them (reviewed in Ruuskanen et al., 2014; Hashmi et al., 2015).

The dietary pathway of trace elements, including toxic ones, to the body of laying female birds and their eggs has been intensively studied in recent years (including studies on metal accumulation in the eggs of poultry; Hashmi et al., 2015). Be this as it may, no detailed investigations have been carried out to relate the properties of the biogeochemical environment to trace element levels in avian eggshells based on exact locations or on the sampling of soil data at large spatial scales under natural conditions (discussed in Ormerod and Rundle, 1998; Ruuskanen et al., 2014; Hashmi et al., 2015). Similarly, the variable quality of physicochemical properties of soils are rarely integrated with ornithological data or bird habitat association studies (Wilson et al., 2005; Gilroy et al., 2008; McCallum et al., 2015). Recently, Ruuskanen et al. (2014), using nationwide data on soil metal levels (in 15 populations across Europe), found that eggshell Pb levels in Pied Flycatchers Ficedula hypoleuca (a species of the forest interior foraging primarily on airborne insects) were positively correlated with soil Pb. However, as the authors themselves stated, soil data of this type did not reflect small-scale variations in soil metal levels, e.g. originating from point sources or due to local biogeochemical variability (cf. Shabasko et al., 2014; Hashmi et al., 2015). It similarly, previous studies rarely examined the relationship between soil properties and the breeding distribution of terrestrial birds using soil data at a resolution (grid square) of 10 km (Gimona and Brewer, 2006; McCallum et al., 2015). Linking low-resolution soil data (in particular, such as those at the nationwide level) is probably an ineffective way of discovering the relationship between soil properties and ecologival/avian data, because detecting the effects of some soil properties (such as its moisture) requires a higher resolution, for example, of 1 km (Gimona and Birnie, 2002). Consequently, it would be desirable to look a posteriori at the dependence of avian data on soil quality at a different spatial resolution.

Typical soil-invertebrate-feeding bird species, such as the Rook Corvus frugilegus, are particularly vulnerable to high doses of toxic chemicals ingested through their food items containing local soil (Malming, 1973; Pinowski et al., 1983; Beyerbach et al., 1987; Ortowski et al., 2012; see Chapman, 2016 for discussion). The Rook is a typical ground-foraging, colonial, omnivorous corvid species, broadly distributed across Eurasia (Haffer and Grull, 1993; Cramp, 1998), where its breeding is heavily dependent on agricultural activities (Kasparykowski, 2003, 2007) and the availability of soil invertebrates associated mostly with moist, loamy soil (Gimona and Brewer, 2006). The present study, based on the framework outlined above, involved the analysis of an extensive dataset relating to the monitoring of soil quality (acidity, levels of elements and organic matter, particle size distribution) throughout Poland in order to predict the concentrations of five trace elements, including three essential metals (Cu, nickel [Ni] and Zn), and two non-essential ones (Cd and Pb) in Rook eggshells from 42 breeding colonies. The data on eggshell metal levels were obtained from our previous studies (Ortowski et al., 2010, 2014a,b). They showed that all five trace elements occurred in Rook eggshells at levels of ecotoxicological concern mostly in samples from urban rookeries, and that there were no significant differences in eggshell metal levels between the regions of Poland with more (western) and less (eastern) intensive agriculture. In those studies, however, we did not make any direct observations linking soil metal levels with eggshell metal levels. In the present work, our major goals were to test: (1) whether soil properties, including soil metal levels, affect eggshell metal levels, and (2) whether there are any differences in the explanatory power of soil data at the various spatial resolutions used as correlates of trace element levels in the shells of Rook eggs. We acquired soil data at four different distances (5, 10, 15 and 20 km) around each rookery, and then correlated them with eggshell metal levels. We expected to find some differences in the effects of soil properties on eggshell metal levels: these are primarily a function of trace element bioavailability with respect to soil acidity, particle size or organic matter/metal levels in the various regions of the study area. On the other hand, associating variations in soil properties with eggshell metal levels is not a straightforward matter, because only a small proportion of the trace elements ingested by females (e.g., together with the contaminant medium: soil particles present in the guts of invertebrate prey items such as earthworms or Coleoptera larvae and/or ingested grit particles) is presumably deposited in eggshells, and some portion of soil/grit particles is regurgitated in pellets (Haffer and Grull, 1993; Czarnecka and Kitowski, 2013; Czarnecka J., unpubl. data). In natural conditions the levels of some toxic elements (e.g. Cd and Pb) can be several times higher in the eggshells of birds than in the whole of the invertebrate prey items they ingest (e.g. Simonetti et al., 2015). Furthermore, metal levels in female bodies may represent the accumulation of these elements over a longer time span, i.e., at both the wintering and breeding grounds (cf. Hashmi et al., 2015). Thus, by determining the relationship between metal levels in soils and eggshells, the results of our analysis ought to contribute to a better understanding of the basic functional ecophysiological relations between a changing environment and the reproductive biology of birds.
2. Material and methods

2.1. The Rook study

In the present study we used our previously published data on trace element levels in Rook eggshells (Ortowski et al., 2010, 2014a). The eggshells (in both this and our earlier works) were collected across Poland in the spring of 2005 in 43 rookeries, ranging in size from 5 to 480 nests (Fig. 1). We picked up the post-hatch eggshells (at least two shell fragments from two different eggs) from the ground beneath the nests.

We provided a detailed description of the chemical analysis of eggshells and validation of the certified reference material in our earlier papers (Ortowski et al., 2010, 2014a). Briefly, since the amounts of eggshell collected in some rookeries were rather small, the metal concentrations for each colony were determined using just two different eggshells or their fragments. In practice, large pieces were used to ensure that the samples had come from different eggs. Remnants of membranes and visible external dirt were removed from the eggshells before these were stored in glass containers for heavy metal analysis. The chemical analyses were performed at the Department of Hydrobiology and Aquaculture of the Wrocław University of Environmental and Life Sciences by three of the co-authors of this paper (WD, PP, RP). Prior to the chemical analysis, all the eggshells were rinsed twice with water containing detergent and then air-dried. They were then mineralised in a mixture of nitric and hydrochloric acid in a high-pressure microwave digestion system (MARS-5; CEM, Matthews, NC). Atomic absorption spectroscopy (SpectAA FS220; Varian, Palo Alto, CA) was used to determine metal concentrations. The measurement process was validated using DORM-3 (fish protein) reference material, provided by the National Research Council of Canada Institute for National Measurement (Ottawa, Ontario, Canada). All the metal concentrations were expressed in milligrams per kilogram (mg kg⁻¹; ppm) of dry mass (d.w.) with an accuracy of two decimal points.

2.2. Soil data: large scale national monitoring of soil quality in Poland

In order to evaluate the effect of the physical properties and chemical composition of soil on trace element levels in Rook eggshells, we used data representative of the extensive nationwide monitoring of soil quality (NMSQ) conducted in Poland in 1995–1997 (Terelak et al., 1997). Importantly, levels of trace elements in soil in Poland generally remained unchanged during the subsequent 15 years (Siebielec et al., 2012). NMSQ was supervised by the Institute of Soil Science and Plant Cultivation (IUNG), Pulawy, Poland (Terelak et al., 1997). Within the framework of this programme, soil samples were collected from 43 792 georeferenced locations representing agricultural land across the entire country. On average, one location represented 200 ha of farmland. The sample at each location consisted of 20 individual topsoil subsamples (0–20 cm depth) collected from a 100 m² (10 × 10 m) square. The NMSQ methodology is described in detail by Terelak et al. (1997). Briefly, soil samples were air-dried, crushed and sieved through a 1 mm mesh prior to analysis. The fundamental soil properties analysed included pH, particle size distribution and total organic matter. Soil pH was measured in a slurry with a 1:2 v/v soil:1M KCl ratio. Particle size distribution was determined using a hydrometer (Gee and Bauder, 1986). Soil organic matter (OM) was measured by the Tiurin method after hot digestion of the sample with potassium dichromate (mineral soils) or by loss on ignition in a muffle furnace at 480 °C within 16 h (organic soils). Total metal (Cd, Pb, Ni, Cu, Zn) levels were analysed by atomic absorption spectrometry following the hot aqua regia sample digestion protocol (digestion in a mixture of concentrated nitric and hydrochloric acids, followed by refluxing in 3 M hydrochloric acid) (McGrath and Cunliffe, 1985). NIST 2709 and 2711 (National Institute of Standards and Technology) reference materials were used for the quality control of soil trace element measurements. For statistical purposes we used the following soil variables: metal concentrations in soil expressed as mg kg⁻¹ of dry weight accurate to two decimal points, soil pH, organic matter content OM (%), and the contents of two particle fractions: <0.02 mm – F3 and clay <0.002 mm – F4 (both as %).

2.3. Data analysis

We used ArcGIS 9.2 and Statgraphic 5.0 software to integrate georeferenced NMSQ soil data with eggshell element concentrations. From the NMSQ database we selected 3724 records from the whole of Poland (soil data monitoring points) within a distance of 20 km around 42 active rookeries. In comparison with our previous study of metal concentrations in Rook eggshells determined for 43 breeding colonies in Poland (Ortowski et al., 2010, 2014a,b), the present analysis omitted the data for one rookery because no soil data were available for it. Then we determined soil data (average values) within four distances at 5 km, 10 km, 15 km and 20 km around each of these 42 rookeries (Table S1). To calculate soil data for these rookeries we used, on average, 1–19 sampling points per colony (436 in all) within a distance of 5 km, 18–32 sampling points per colony (1310 in all) within a distance of 10 km, 33–84 sampling points per colony (2447 in all) within a distance of 15 km and 76–130 sampling points per colony (3724 in all) within a distance of 20 km. The rationale for the selection of these four distances was dictated by the desire to achieve a high spatial convergence between soil data and eggshell data, which was subsequently assessed in the statistical analysis. To date, however, the foraging distance of adult Rooks during the brood rearing period was found to be < 3 km (Mason and MacDonald, 2004; Macdonald and Whelan, 1986; Kasprzykowski, 2003). Since NMSQ data were sampled in agricultural areas away from typical urban areas (where 14 of our rookeries were situated; Ortowski et al., 2010, Ortowski et al., 2014a, b) we did not distinguish between urban rookeries and rookeries on agricultural land away from typical urban areas (where 28 of our rookeries were situated; Orłowski et al., 2010, Orłowski et al., 2014a, b).
(mostly located on the outskirts of urban areas) and rural rookeries in the main analysis (Fig. S1). However, to test whether the soil data were better correlated with the eggshell samples taken only from rural areas, we also provide the results of this complementary analysis for 28 rural rookeries (see Supplementary data).

All the statistical analyses were performed with Statistica 7.0 (StatSoft, 2006) and Excel. A probability of \( P < 0.05 \) was considered statistically significant.

In the first step of our analysis, we compared the distribution pattern of the five target trace elements between eggshells and soil measured for these same rookeries. For this purpose we used the concentrations of elements determined for the smallest distance of 5 km around rookeries. We applied the \( t \)-test to dependent samples to compare the concentrations of trace elements measured in eggshells and in soil around the same colonies.

Then we tested whether any differences existed in the predictive power of soil data compiled for four different distances around an individual rookery used as correlates of trace element contents in Rook eggshells. Owing to the strongly correlative nature of the physical properties of soil (i.e. there are significant relationships between soil acidity, OM and the contents of fractions F3 and F4), we used PCA (with varimax normalised factor rotation) to identify the rookery-related spatial associations between these properties.

We calculated four different PCAs using Statistica 7.0 (StatSoft, 2006) across the four distances around all 42 rookeries. Factor loadings (PCs) with an eigenvalue \( >1 \) were considered to account for a significant contribution to the total variance according to the latent root criterion (Hair et al., 1998).

We used a general linear model (regression analysis in GLM module in Statistica 7.0; StatSoft, 2006) to assess the effects of the physical properties of soil (PCs) and the chemical composition of soils on eggshell trace element concentrations. We ran four different multivariate GLMs at 5 km, 10 km, 15 km and 20 km for each trace element. In the GLMs for 5 and 10 km we introduced two variables (the concentration of an element in the soil and one PC1) and three for 15 km and 20 km (the concentration of an element in the soil and two soil properties — PC1 and PC2). To improve the normality of the distribution and equalising variance, as well as the linearity between the response and explanatory variables, we log-transformed the eggshell concentrations of Cu, Zn and Pb and soil levels of Cd at all four distances around the rookeries so as to meet the assumptions of linear modelling prior to analysis; the distribution of all the remaining concentrations of soil/eggshell elements was normal (examined using the Kolmogorov-Smirnov test). The relationships between soil data and eggshell trace element concentrations (where appropriate on log-transformed data; percentage data were square-root transformed), analysed in a univariate manner using the Pearson correlation coefficient (Table S2, S4–S5), are given in the Supplementary data (Tables S1–S5).

3. Results

The initial comparison of element distribution indicated that only the Pb level was consistently higher in soil samples than in eggshells across all 42 rookeries (Fig. S1; Table S1). The distribution pattern of the remaining elements was much more variable. The concentrations of Cu, Zn and Ni were higher in soils than in eggshells in most of the rookeries, i.e. 35/42, 39/42 and 41/42 colonies, respectively, whereas eggshells had higher Cd levels than soils in 41/42 rookeries (Fig. S1; Table S1).

Taking all 42 rookeries into account, paired comparison (\( t \)-test for dependent samples) of trace element concentrations measured in eggshells and soils within a distance of 5 km around the rookeries showed that only the Cu level did not differ between these two samples (\( P \geq 0.494 \)). The concentrations of the other elements varied significantly between them (Fig. S1; Table S1); that of Zn was on average 3.1-fold higher in soils than in eggshells (\( P < 0.0001 \)), while those of Ni and Pb were on average 4.8-fold and 5.1-fold respectively in soil samples than in eggshells (\( P < 0.0001 \), in each case). In eggshells only Cd concentrations were higher (by c. 2.2-fold) than in soils (\( P < 0.0001 \)) (Fig. S1; Table S1).

3.1. Effects of physical properties and chemical composition of soils on eggshell trace element concentrations

PCA of the physical properties of the soils around the 42 rookeries (Tables S1–S5) yielded all components with eigenvalues \( >1 \), which explained from 26% to 72% of the variance, although there were slight differences in the groupings of some of these properties (Table 1). For the distance of 5 km around the rookeries, only one principal component (PC1) was derived grouping all the soil properties (pH + OM + F3 + F4); for 10 km PC1 was obtained for three soil properties (pH + F3 + F4). For the two larger distances around the rookeries (15 and 20 km), two principal components were derived: PC1 (pH + F3 + F4) and PC2 (OM) (Table 1).

GLMs testing the effect of physical properties and levels of trace elements in soils on trace element concentrations in eggshells showed eight statistically significant fits of full models for three elements: Cu, Cd and Pb (Table 2). In contrast, Zn and Ni concentrations in eggshells were independent of their levels in the soils near the rookeries (Table 2). Interestingly, across all four distances around the rookeries (5, 10, 15 and 20 km) only the eggshell Cd concentration was significantly affected by the physical properties of soils (only PC1, a negative effect; Table S2) and Cd soil content, a positive effect (Fig. 1). Moreover, the percentage of variability explained by the fits of these four full models (\( R^2 \)-values) varied only slightly, between 31% and 36%; these values were higher than the fits of other elements (Table 2). Similarly, at all four distances around the rookeries there was a statistically significant positive relationship between the Cd levels measured in eggshells and soils (Table S3).

There are two significant GLM fits for eggshell Cu concentrations: at 5 and 10 km (Table 2). Furthermore, at all four distances around the rookeries univariate analysis yielded statistically significant positive relationships between Cu levels measured in eggshells and soils (Fig. 2; Table S2), and also between eggshell Cu concentrations and certain soil properties: PC1 at 5 km; soil acidity, F4 and PC1 at 10 km (Fig. 2); F4 and PC1 at 15 km, and F4 at 20 km (Table S4).

GLMs of eggshell Pb concentrations yielded significant fits for the two largest distances, i.e. 15 and 20 km (Table 2). In both these models we detected a positive effect of soil Pb and a negative effect of soil PC1 (Table 2). Similarly, in the 15 and 20 km models we found statistically significant positive relationships between the Pb levels measured in eggshells and soils (Fig. 2; Table S4). Lastly, we found a statistically significant negative relationship between eggshell Pb concentrations and soil acidity at 5 km around the rookeries (Fig. 2; Table S5).

4. Discussion

Two major results emerge from our investigation of a large dataset of nationwide soil quality monitoring and levels of trace elements in Rook eggshells. Soil properties, including soil metal levels, affect eggshell metal levels, although the extent to which this occurs depends on the target element and the spatial resolution of soil data. In other words, our study has demonstrated that soil properties are useful for predicting actual levels of trace elements in avian eggshells. The significantly higher level of Cd in
eggs than in soils indicates that the former are of particular importance for female birds as regards the excretion of this inorganic pollutant (Burger, 1994; Hashmi et al., 2015; Simonetti et al., 2015). According to our findings, the spatial resolution of soil data has a relatively small influence on the model prediction of the physicochemical properties of soil and trace elements in Rook eggshells; discrepancies were identified only in the case of Pb. Our analysis demonstrated that across all four distances, i.e., 5, 10, 15 and 20 km around the rookeries, only eggshell Cu and Cd were positively correlated with their soil contents, whereas eggshell Pb was correlated with the soil Pb level at the two largest distances (15 and 20 km) around the rookeries. These new and important results fill the gaps in our knowledge about the functional relationship between metal levels in soils and avian eggshells at large spatial scales. The major explanation of our findings is metal transfer along the food chain, i.e., the direct or indirect (through swallowing of prey) ingestion of local soil and/or grit particles by female Rooks. Grit particles (presumably also some portion of soil; see Graphical abstract) are often ingested by adult and nestling Rooks (which was confirmed by dietary analyses of both regurgitated pellets and stomach contents; Haffer and Grull, 1993; Orłowski et al., 2009; Czarnecka and Kitowski, 2013; Czarnecka J., unpubl. data), both for mechanical breakdown of food and presumably (in nestlings) as a source of minerals for skeleton formation and other physiological needs (discussed in Orłowski et al. 2009). Moreover, we reported previously that Rook nestlings (with high Cd and Pb levels in their tissues; they came from several breeding colonies in north-eastern Poland where we also collected eggshells used in this study; cf. Orłowski et al., 2012) showed a significant positive relationship between the number of animal food items and the Cd level in the kidneys, as well as a negative relationship between the number of plant items (mostly cereal seeds) and the Pb level in the liver, and between the number of grit particles and the Pb level in the kidneys (Orłowski et al., 2013). This suggested the substantial bioavailability of Cd from animal food items and a low level or reduced gastrointestinal absorption of Pb from plant food in Rook nestlings (Orłowski et al., 2013). To conclude, further large-scale studies need to be carried out in order to assess the relationship between the levels of non-essential elements and other agricultural-related contaminants at various trophic levels of the food pyramid (soil—prey, including frequently consumed cereal seeds and the gut contents of prey items) with regard to the Rook, its eggs and reproductive performance.

Interestingly, the results of our analysis of the relationships between soil properties and the eggshell metal levels given in this paper tally with those of an earlier investigation, based on a large set of toxikinetic studies, of the bioaccumulation of the same metals in soil organisms (the staple diet of Rooks), reviewed in detail by Ardestani et al. (2014). In particular, the soil concentrations of all five metals examined in our study were positively correlated with their levels in soil invertebrates, although the uptake of some metals like Zn may have been slower (Ardestani et al., 2014). Furthermore, soil pH and OM content were negatively correlated with the concentrations of Cd, Pb and Cu in soil invertebrates, but not with Zn (Ardestani et al., 2014). In this sense, of particular importance to explain is obtained in our study a positive relationship between OM and eggshell Cu (see Table S4), which do not agree with the pattern of bioaccumulation of this metal in soil invertebrates (cf. Ardestani et al., 2014). This discrepancy can most likely be explained by the particularly high levels of Cu in eggshells from some rookeries in areas of western Poland (see Table S1) naturally rich in Cu where this metal is mined (cf. Orłowski et al., 2010). To date, the more significant relationships between soil metal and eggshell metal levels obtained in our study, compared to the earlier study of Ruuskanen et al. (2014) (where such a link was detected only for Pb), suggests a stronger association between the Rook and soil environments and/or more effectively reflect the soil metal-eggshell metal relationship at a finer resolution of soil data. Similarly, a previous study relating the content of Pb, Cu and Zn in soil to the concentrations of these elements measured in entire eggs of hens Callus domesticus reared in open conditions with access to local soil in 59 sites across Belgium showed that only the Pb concentration was correlated among these samples (Waegeleurs et al., 2009). Therefore, it is highly possible that the significant relationship obtained in our study between the levels of Cu, Cd and Pb measured in soil and eggshells resulted from the relatively large concentration ranges of these elements in soil and other physical soil properties measured around all the rookeries we analysed. In particular, our analysis of the relationships for these elements, repeated for only rural rookeries where we recorded lower concentrations of Cu, Cd and Pb (see Fig. S1), did not yield any significant relationship (Table S5). This might also be explained in part by a geographical bias in the distribution of the rural rookeries, which were situated predominantly in eastern Poland, where the concentrations of all the target elements were significantly lower than in the western part of the country (see Table S3). Consequently, even though our previous simple urban–rural classification of Rook breeding colonies explained the differences in eggshell metal levels and seemed to outweigh the underlying biogeochemical characteristics of soil, the use of soil properties as correlates of eggshell characteristics may be generally ineffective when samples of eggshells and soil are small and/or exhibit little variability. Hence, for analyses of soil-bird relations, we evidently need to generate soil data of a greater resolution in order to capture the subtle differences in soil properties. The considerable variability in soil properties across the country is reflected in the significant regional differences in soil quality between western and eastern Poland (see Table S3), which corresponds closely with the geochemical and historical differences in soil formation after the last Pleistocene glaciation (Dzięciołowski and Tobolski, 1982).

Our other important finding is that the physical properties of soil (primarily the increase in pH) negatively affected eggshell Cd and Pb concentrations. We found that eggshell Pb was correlated with soil Pb at the two greatest distances (15 and 20 km) around the rookeries, and that only at the shortest distance (5 km) was the soil pH negatively correlated (univariate analysis) with eggshell Pb concentration (see Fig. 2 and Table S4). This is because the variations explained by PC scores decrease with increasing distance from the rookeries. This, however, has a limited influence on the GLM results of most eggshell elements apart from Pb. It is possible that our samples of soil pH and soil Pb do not reflect the real local

Table 1 Component values and factor loadings of PCA of four physical properties of soil: acidity, pH; organic matter content, OM; the content of fraction <0.02 mm, F3 and fraction of <0.002 mm, F4 (% clay) determined on the basis of large-scale national monitoring of soil quality within four distances (5, 10, 15 and 20 km) around 42 breeding colonies of the Rook Corvus frugilegus in Poland. The figures in bold indicate the variable for which each factor exhibited the greatest variability.

<table>
<thead>
<tr>
<th>Soil characteristic</th>
<th>Distance around colony</th>
<th>5 km</th>
<th>10 km</th>
<th>15 km</th>
<th>20 km</th>
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<tr>
<td></td>
<td>PC1</td>
<td>PC1</td>
<td>PC1</td>
<td>PC1</td>
<td>PC2</td>
</tr>
<tr>
<td>pH</td>
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<td>0.745</td>
<td>0.786</td>
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<td>OM</td>
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<td>0.531</td>
<td>0.882</td>
<td>0.953</td>
<td>0.036</td>
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<td>F3</td>
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<td>0.890</td>
<td>0.888</td>
<td>0.263</td>
<td>0.920</td>
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<tr>
<td>F4</td>
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<td>0.947</td>
<td>0.942</td>
<td>0.237</td>
<td>0.960</td>
</tr>
<tr>
<td>Eigenvalues</td>
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<td>2.30</td>
<td>1.10</td>
<td>2.32</td>
</tr>
<tr>
<td>Variation explained</td>
<td>72%</td>
<td>63%</td>
<td>58%</td>
<td>28%</td>
<td>58%</td>
</tr>
</tbody>
</table>
values of these soil properties around rookeries. Meanwhile, it was observed that the properties of the soils under Rook nests in rookeries (woodlots) differ significantly from those at the control sites in the adjacent arable land (Krółak et al., 2014; Borkowska et al., 2015). These differences were attributable to changes in soil properties owing to the accumulation of Rook faeces at the nesting sites, as indicated by the higher soil acidity and organic matter content (Borkowska et al., 2015) and higher concentrations of soil Cu, Zn and Cd (Krółak et al., 2014). Because adult Rooks gather their nest material, and presumably forage at the same time, within the

Table 2

Results of multivariate GLMs testing the effect of physical properties (PC1 and PC2) and content of various trace elements in soil on eggshell trace element concentrations in Rooks Corvus frugilegus from 42 breeding colonies in Poland. Soil data from PCA (see Table 1 for more details) determined within four distances: 5 km (PC1: pH + OM + F3 + F4); 10 km (PC1: pH + F3 + F4); 15 km and 20 km (PC1: pH + F3 + F4; and PC2: OM). F-value and accompanied P-value used to assess the effect of an individual variable at *P ≤ 0.05, **P < 0.01, ***P < 0.001; summaries of full models, which meet the threshold of statistical significance, are given in bold.

<table>
<thead>
<tr>
<th>Eggshell element</th>
<th>Distance around colony</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 km</td>
</tr>
<tr>
<td>Cu</td>
<td>(Soil Cu) 5.27*;</td>
</tr>
<tr>
<td></td>
<td>(Soil PC1) 0.13;</td>
</tr>
<tr>
<td><strong>R² = 0.201, F = 4.80, P = 0.014</strong></td>
<td><strong>R² = 0.159, F = 3.69, P = 0.034</strong></td>
</tr>
<tr>
<td>Zn</td>
<td>(Soil Zn) 0.78;</td>
</tr>
<tr>
<td></td>
<td>(Soil PC1) 0.01;</td>
</tr>
<tr>
<td><strong>R² = 0.034, F = 0.67, P = 0.518</strong></td>
<td><strong>R² = 0.056, F = 1.16, P = 0.323</strong></td>
</tr>
<tr>
<td>Ni</td>
<td>(Soil Ni) 2.79;</td>
</tr>
<tr>
<td></td>
<td>(Soil PC1) 5.22*;</td>
</tr>
<tr>
<td><strong>R² = 0.121, F = 2.61, P = 0.087</strong></td>
<td><strong>R² = 0.001, F = 0.01, P = 0.993</strong></td>
</tr>
<tr>
<td>Cd</td>
<td>(Soil Cd) 12.28***;</td>
</tr>
<tr>
<td></td>
<td>(Soil PC1) 9.47***;</td>
</tr>
<tr>
<td><strong>R² = 0.312, F = 8.61, P = 0.0008</strong></td>
<td><strong>R² = 0.357, F = 10.85, P = 0.0002</strong></td>
</tr>
<tr>
<td>Pb</td>
<td>(Soil Pb) 3.15;</td>
</tr>
<tr>
<td></td>
<td>(Soil PC1) 2.49;</td>
</tr>
<tr>
<td><strong>R² = 0.089, F = 1.86, P = 0.170</strong></td>
<td><strong>R² = 0.121, F = 2.68, P = 0.081</strong></td>
</tr>
</tbody>
</table>

Fig. 2. Concentrations of Cu, Pb and Cd in Rook Corvus frugilegus eggshells plotted against different physical properties (a–b) and trace element levels in soil (c–e) determined at various distances around breeding colonies of the species in Poland; see Tables S2–S3 for more details.
area of the breeding colony (Coombs, 1960; Roskaft, 1983), enhanced (or impaired) transfer of metals from soil to females at these sites during the early nesting period is possible. Another explanation is that soil pH is of paramount importance compared to soil Pb, acting as a major factor limiting the bioavailability of Pb to laying females at the breeding location. The latter explanation largely agrees with the results of prior studies on the bioaccumulation of Pb in avian eggshells (Ruuskanen et al., 2014).

Admittedly, the soil data used in our analysis probably explain only part of the variability in the actual levels of Cu, Cd and Pb in eggs. The large remainder of this variability, resulting from the ingestion of invertebrate prey with different trace element levels (i.e., after application of agrochemicals or a variable volume of soil in their digestive tract; Pinowski et al., 1983; Chapman, 2016) or female condition as indicated by her reserves of calcium (Reynolds and Perrins, 2010) or other metals (in particular Pb and Cd; Dauwe et al., 2005) is yet to be explored. This could explain the observed variable distribution patterns of the concentrations of most elements between soil and eggshells (except for Pb, which was consistently higher in soil than in eggshells in all the rookeries). Furthermore, because we found that eggshell Zn and Ni concentrations were independent of their soil levels, some physiological mechanism responsible for the limited transfer of these elements along the food chain to female Rooks and their eggs is possible, which agrees with the results of earlier rare avian studies (e.g., Roodbergen et al., 2008). Finally, even though our analysis confirmed strong mutual associations among the different physical properties of soil, confirmed both by PCA (see Table 1) and univariate manner (see Table S2), all these properties except for soil acidity contributed in a very limited way, if at all, to the transfer of trace elements to Rook eggshells.

To conclude, our results imply that, during their short stay in their breeding grounds, female Rooks selectively take up Cu, Cd and Pb from the environment and deposit these elements in eggshells. This appears to confirm that Rooks (like most passerines) are characterised by a short-term allocation of maternally derived substances and contaminants primarily from the current food intake; this species should therefore be treated as income breeders, i.e. species that use their current food intake for reproduction (sensu Ward and Bryant, 2006; Van Dyke et al., 2013). More importantly, however, owing to the probable absorption of some portion of trace elements from eggshells by developing avian embryos (Ortowski et al., 2016), metal levels in Rook eggshells measured in eggs at hatching (i.e., without embryonic development) may well be higher than in the post-hatch shells used in the present analysis. Because the levels of several trace elements in Rook eggshells were of ecotoxicalological concern, including non-essential ones like arsenic (As), Cr, Cd, Pb and Cu, further studies to assess the pressure of inorganic contaminants on embryonic development in this and other soil-invertebrate feeding birds are needed.

The patterns and factors governing the bioaccumulation of metals in soil invertebrates and eggshells appear to be coincident, which strongly suggests a general similarity in the biochemical pathways of elements at different levels of the food pyramid. The spatial resolution of soil data exerts little influence on model predictions of the physicochemical properties of soil and eggshell trace elements in Rooks. The ongoing acidification of arable soil owing to natural processes in sandy soils and the often intensive fertilisation and nitrification of the land can accelerate the bioavailability of toxic elements to laying females and their eggs. This clearly implies that certain agricultural practices, such as timing, is of particular importance as regards reducing the transfer of inorganic contaminants to females of soil-invertebrate feeding birds and their eggs. It should be highlighted that even though the application of agricultural lime is recognised as beneficial for soil-invertebrate feeding birds, mostly due to the increase in soil pH and the higher abundance of some soil invertebrates (cf. McCallum et al., 2015), some trace elements (including Pb, Cu, Zn and Cd) can occur in lime fertilisers at concentrations far in excess of their levels in soils (Marek, 1990; McBride and Spiers, 2001). The application of certain lime agents to soil thus has a downside: the input of surplus bioavailable trace elements into terrestrial food webs, as well as decreasing the numbers and diversity of certain invertebrate groups (cf. Vickery et al., 2001).

We recommended further studies integrating as yet wholly unexplored relationships between soil properties determined at large spatial scales and different aspects of avian ecophysiology and reproduction (including investigations of egg composition), which might be useful in explaining patterns in the occurrence, physiology and perhaps decline of avian populations in human altered ecosystems.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.10.048.

References
