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Leaching of Metals from Steel Slag and Their Ecological Effects on a Marine Ecosystem: Validating Field Data with Mesocosm Observations

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Abstract: Steel slag is being used worldwide for a variety of applications, among which is underwater dyke reinforcement. In the present study the leaching and bioaccumulation of 18 inorganic compounds from basic oxygen furnace (BOF) steel slag were monitored in marine experimental ecosystems (mesocosms) for 12 wk. Triplicate mesocosms were installed at 2 refreshment rates, one reflecting the situation in the Oosterschelde estuary where BOF steel slag was applied and the other at a 35 times lower rate. Vanadium in both water and biota turned out to be the best tracer for the presence of BOF steel slag in the mesocosms. The mesocosm data helped to interpret the results of a 4-yr field sampling program in the Oosterschelde estuary where no elevated levels of vanadium in water or biota were found near locations where steel slag was applied. Also, no ecological impact could be established in the field, which was in line with the observations in the mesocosms. The present study shows the added value of a tailor-made mesocosm study for realistic risk assessment and provides support for applying this tool as a basis for designing efficient field monitoring programs. *Environ Toxicol Chem* 2021;40:2499–2509. © 2021 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Metal accumulation; Heavy metals; Water quality; Benthic macroinvertebrates; Metal bioavailability; Steel slag; Field monitoring; Vanadium

INTRODUCTION

Steel slag as building material

Steel slag, a nonmetallic by-product of the iron- and steelmaking process, is being used worldwide for a variety of applications (Proctor et al. 2000; Motz and Geiseler 2001; Fisher and Barron 2019), among which is as aggregate in engineering projects like road construction or reinforcement of river banks. The societal benefit of the use of such industrial by-products is that it reduces the use of natural resources (Motz and Geiseler 2001). Three types of slag result from the steel industry, which are named for the processes from which they are produced: blast furnace (BF) iron slag, basic oxygen furnace (BOF) steel slag, and electric arc furnace (EAF) steel slag. The main components of all slag are silica, calcium, and alumina and magnesium oxides; but in BOF and EAF slag the concentrations of iron and manganese are substantially higher than in BF slag (Proctor et al. 2000). Apart from the stability of the material, which is relevant from the engineering perspective, the leaching behavior of steel slag is important to assess potential environmental impact (Proctor et al. 2000; Fisher and Barron 2019). The suitability of steel slag in waterworks is usually stated by fulfilling the requirements of national and/or international standards and regulations (Motz and Geiseler 2001). In The Netherlands this is regulated by the Soil Quality Decree of the Food and Agriculture Organization of the United Nations and the related Soil Quality Regulation (Food and Agriculture Organization of the United Nations 2012). Emission standards

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are based on maximum permissible addition and are generic for the use of building materials in Dutch surface waters, where water flows ranging from 0.15 to 25 m^3/s are considered (Verschoor et al. 2006). Also named Linz-Donawitz (LD) slag, BOF slag has been tested according to this regulation and is certified for use as erosion protection material in Dutch waterworks, along riverbanks and dykes.

Use of steel slag in Oosterschelde estuary

Between 2009 and 2018, BOF slag has been applied for underwater dike reinforcement in the Oosterschelde estuary. This 350-km^2 tidal estuary, located in the southwestern part of The Netherlands (Smaal et al. 2009), was designated a national park in 2002 and received the status of European "Natura 2000 area" in 2010. Besides supporting recreational activities which are allowed under regulation (Ministry of Agriculture Nature and Food Quality 2004, 2010), fisheries are the most important function, with mainly mussel and oyster farms and cockle fishing. The use of steel slag in this estuary caused great public concern, especially about the impact that potential leaching of heavy metals might have on the local ecosystem. To overcome the concerns, an annual field monitoring program was conducted between 2009 and 2014, in which species diversity and heavy metal concentrations in selected species were determined in areas where steel slags had been applied and at reference locations. Metal concentrations showed high variation both between the monitored species and between monitoring years. Mercury, cadmium, selenium, and molybdenum were sometimes detected in biota in what seemed higher concentrations compared to other samples from the monitoring program; but the data showed no correlation with the presence of steel slag in the area. Nor was it possible to establish a link between heavy metal concentrations or the presence of steel slag and the diversity of the local benthic community (Tangelder et al. 2015).

To obtain a more complete picture of the potential effects of steel slag on the chemistry and ecology in this marine system, a study was developed in marine experimental ecosystems (mesocosms) that hold a community representative of the Oosterschelde estuary. The advantage of such a study over laboratory tests is that in mesocosms a more realistic situation can be created, taking into account interactions between species and their environment. The advantage of a mesocosm study over field monitoring is that test conditions can be controlled, replicated, and compared with replicated control situations under the same conditions.

The tests were performed in flow-through mesocosms at 2 refreshment rates, one being realistic for the field situation and a much lower rate that can be considered a nonrealistic worst-case situation. The mesocosm results will indicate what the environmental response to steel slag looks like and, as such, help interpretation of the data from the field monitoring program.

In addition to steel slag, natural quarry rock that is also traditionally applied for dike reinforcement in the Oosterschelde was tested in the same way. Quarry rock can also leach heavy metals (Taha et al. 2019) into the water column. Pebbles were used as reference material, assuming that they do not leach heavy metals.

Research goals

The aim of the present study was to get a clear picture of 1) what inorganic compounds leach from BOF slag in a marine environment, 2) which of these inorganics accumulate in biota, and 3) the potential impact of BOF slag on the ecosystem. This insight should clarify if the fact that no ecological impacts or clear chemical traces were observed during field monitoring indeed indicated the lack of effect of steel slag or was due to inadequate sampling.

MATERIALS AND METHODS

Mesocosm preparation

The 18 mesocosms used for the present study were created in polyester tanks with a diameter of 2 m, a depth of 1 m, and a volume of approximately 2.5 m^3 and were located outdoors next to our laboratory in the city of Yerseke, The Netherlands, at the border of the Oosterschelde estuary. After approximately 20 cm of sandy sediment was added, the tanks were filled with seawater from the Oosterschelde containing a natural plankton community. The water used to fill and refresh the mesocosms came from a pipe directly from the Oosterschelde by means of a permanent pumping system that also provides seawater to the local shellfish industry. The water was extracted near the test facility and at least 15 km away from locations where steel slags have been applied. Half of the mesocosms received refreshment water with a "high" flow rate of 350 L/h. When continuous complete mixing of the water column is assumed, these mesocosms would contain only 1% of the original water after 24 h. This forms a realistic minimum refreshment rate along a dike in the Oosterschelde estuary according to model calculations (Kleissen 2015). The water in the other mesocosms was replaced at a "low" rate of 10 L/h. After 24 h, these mesocosms should still contain 90% of the original water.

An initial community of small benthic invertebrates was introduced in each mesocosm by adding 2 L of surface sediment that had been freshly collected from a tidal flat in the Oosterschelde. Before introduction, the sediment was sieved over a 5-mm sieve to remove larger organisms and coarse materials. It was then mixed and divided into portions of 2 L that were suspended in the water column of each mesocosm, while the water refreshment was stopped until the next morning. After reinstallation of the water refreshment, the mesocosms were allowed to establish for 4 wk before the test materials were introduced. This timing is essential to give planktonic and microbial communities time to adjust to the new situation and to start colonization of the sediment with larvae that are introduced with the water. During this establishment period, larger organisms were introduced. Within the first week, 20 small cockles (*Cerastoderma edule*; shell length 16.4 ± 1.0 mm) and 10 lugworms (*Arenicola marina*; individual wet wt 4.6 ± 2.2 g) were spread in the half of the sediment that was not going to be covered with the test material. To minimize the change of individuals moving to the other side, the sediment surface was divided into 2 halves by a 15-cm-high polycarbonate strip that was pushed into the sediment until sticking 1 to 2 cm above the sediment floor. In the same period, 10 individual stems of bladder wrack (*Fucus vesiculosus*; 18.9 ± 9.2 g wet wt each) were introduced in each mesocosm. The stems were attached to a rope that was positioned above the water. By tightening the rope, the plants were lifted above the water surface for approximately 4 h/d to simulate the low-tide period that is essential for the condition of this species. Also, 10 polyethylene baskets, all containing 15 small mussels (*Mytilus edulis*; shell length 34.2 ± 3.0 mm), were introduced at half the water depth in the water column of each mesocosm. After 3 wk, 7 d before application of the test materials (day –7), periphyton was visible on the mesocosm walls and 25 periwinkles (*Littorina littorea*) that feed on periphyton were introduced. Sponges (*Halichondria panicea*) were not directly available and were therefore introduced just before the introduction of the test materials on day 0. Two parts (individual wet wt 37.5 ± 9.4 g) cut from larger specimens were suspended at half the water depth in each mesocosm. All introduced sediment and organisms were collected from sites in the Oosterschelde away from the locations where steel slags have been applied.

Test materials and treatments

Three test materials were used: 1) BOF steel slag, also named LD slag, size range 45 to 180 mm, produced by ArcelorMittal; 2) natural quarry rock, size range 90 to 150 mm, collected near Doornik in Belgium; and 3) pebbles, size range 50 to 150 mm, collected from the North Sea (English Channel area).

The test materials will further be referred to as "slag," "rock," and "pebble." The slag and rock that were used represent the actual material that was applied for dike reinforcements in the Oosterschelde estuary. The pebbles served as a reference material, assuming that they produce the same physical impact in the mesocosms as the slag and rock, though with a minimum predicted impact on the chemical composition of the water.

At the start of the exposure phase (day 0), the test materials were gently introduced in the mesocosms in such a way that half of the sediment surface in the mesocosm was covered with a 10-cm layer of material (~0.2 m^3/mesocosm). Each test material was tested in 6 mesocosms, 3 with a low and 3 with a high refreshment rate. In this way all treatments (test material vs refreshment rate) were created in triplicate. The 6 treatments will further be referred to as "slag-low," "rock-low," and "pebble-low" for treatments with low refreshment rate and "slag-high," "rock-high," and "pebble-high" for treatments with high refreshment rate.

Sampling and analyses

Water characteristics. Water temperature, dissolved oxygen concentration, salinity, and pH in the mesocosms were determined at least once a week by submerging at half the water depth Hach electrodes (LDO101, CDC401, PHC101) and a Dr Lange HT1 for turbidity. At the same time, characteristics of the refreshment water were determined in water samples that were collected directly from the inflow of a mesocosm with high refreshment rate. For the analyses of nutrients and dissolved organic carbon (DOC), a water sample was filtered over 0.45 µm. Analyses of the nutrients nitrate, nitrite, ammonia (together reported as inorganic nitrogen), silicate and orthophosphate were carried out using an Autoanalyzer (Aquakem) with spectrophotometric detection (Netherlands Standardisation Institute 2007). The concentration of nitrate was determined using a flow-through analysis system (Skalar) and spectrophotometric detection (International Organization for Standardization 1996). The water samples for DOC analysis were acidified to pH 2 to 3 with HCl (Merck; Suprapur) and oxygen-purged to remove inorganic carbon. The remaining DOC was analyzed by means of a Shimadzu TOC-V analyzer (International Organization for Standardization 1999).

Biota. Phytoplankton density in the water column was assessed by measuring the chlorophyll-a concentration in water samples by means of a 1 Hz–kuvetten fluorimeter (BBE-Moldaenke). The development of periphyton (sessile algae) was monitored on 3 glass microscope slides (76 × 26 mm) that were placed in the vertical position facing south at approximately 10 cm below the water surface in each mesocosm. After 4 wk, these glass slides with their holders were placed directly in a Biotek synergy HTX multimode plate reader equipped with 440- and 680-nm filters that determined fluorescence units as a proxy for chlorophyll-a concentrations at 70 positions on each glass slide. The median value of the 280 readings from the 4 glass slides per mesocosm was used as an indicator of the biomass of periphyton on the glass slides.

On days 14, 28, 56, and 84, bladder wrack stems were weighed after the water was shaken off; and 2 stems from each mesocosm were stored in polyethylene bags at –18 °C until chemical analyses. On the same days, 2 baskets containing mussels were collected from each mesocosm, survival and shell length were determined, and the mussels were stored in polyethylene bags at –18 °C until chemical analyses. Individual development of the sponges was monitored by determining wet weight and by describing the visual appearance on days 58 and 84. On each of these days, one sponge per mesocosm was stored in a polyethylene bag at –18 °C until chemical analyses.

To avoid disturbance of the system, periwinkles and cockles were only sampled at the end of the present study, after the water was pumped out. Smaller benthic macroinvertebrates were also sampled only at the end of the study. For this, 2 polyvinyl chloride rings, each with a diameter of 30 cm, were pressed in the sediment surface before the water was fully pumped out. The top 5-cm sediment layer within each ring was collected, sieved (500 µm), and sampled for macroinvertebrates, which were then preserved in a 4% buffered formalin solution for further identification and counting at the laboratory. Finally, all pebbles, rock, and slag from the mesocosms were inspected by eye for organisms that were identified up to the species level where possible by the taxonomic experts in our institute.

Chemical analyses. At weekly intervals water samples were collected for analyses of the dissolved fraction of 18 inorganic compounds (Table 1), following the method described in Foekema et al. (2015). In short, 60-mL samples were collected from each mesocosm at half the water depth using a polyethylene syringe. Refreshment water was collected in a precleaned glass beaker directly from the inflow of a mesocosm with high refreshment rate. From this glass beaker also 60 mL was sampled with a polyethylene syringe. A 0.45-µm cellulose acetate membrane filter (25 mm; Whatman) was then mounted on the syringe and the sample purged through. The use of a 0.45-µm filter was based on earlier experiences that filters with smaller pore size (0.2 µm) become clogged rapidly with mesocosm water. After the first 10 mL was discarded, approximately 40 mL of the filtered water was collected in a polyethylene bottle and acidified with concentrated HCl (Merck; Suprapur) to pH <2. The samples were then stored until chemical analysis by means of high-resolution inductively coupled plasma atomic mass spectrometry (ICP-MS; see Supplemental Data, S1.4, for additional information on the method). Quality control was carried out by analyzing a reference seawater sample as a control with each analytical run. The results were checked against the previous results using a control chart. All sample treatments were performed in a laminar flow cabinet to minimize the risk of contamination.

Chemical analyses were also conducted on tissues of bladder wrack, sponges, and mussels. Mussel tissue was collected from the shells with titanium knives in a precleaned sample container. After weighing to determine the average tissue weight per mussel, the sample was homogenized with a titanium dispersing element mounted in a Ultraturrax homogenizer. Sponge tissue was homogenized in the same way. Bladder wrack samples were too solid for this approach and were processed as a whole. All equipment, sample containers, and other materials that came in contact with the samples were prerinsed with an HCl (Merck; Suprapur) solution.

Before chemical analyses, 2 to 5 g of biota sample was digested with 5 mL concentrated nitric acid (TraceMetal™ grade; Thermo Fisher Scientific) for 1.5 h in a heating block at 100 °C. For seawater analysis, all manipulations were performed inside a laminar flow hood using labware prewashed with nitric acid. Where necessary, samples were diluted with 0.1 mol/L nitric acid into the calibration range of the assay.

TABLE 1: List of the 18 inorganic compounds that were analyzed in water and biota in the present study

Compound	Symbol	Compound	Symbol
Aluminum	Al	Lead	Pb
Antimuonium	Sb	Manganese	Mn
Arsenic	As	Mercury	Hg
Barium	Ba	Molybdenum	Mo
Cadmium	Cd	Nickel	Ni
Cobalt	Co	Selenium	Se
Copper	Cu	Tin	Sn
Chromium	Cr	Vanadium	V
Iron	Fe	Zinc	Zn

Samples were analyzed with an Element XR high-resolution ICP-MS (Thermo Fisher). A plasma of 98/2% argon/methane was used to reduce salt deposits on the entrance cone. Quantitation was performed by a 6-point external calibration line containing each element in the relevant concentration range, with tellurium, rhodium, iridium, or thallium as internal standard depending on the analyte. Quality of the biota analysis was monitored with a control chart for As, Cd, Cr, Cu, Hg, Mn, Mo, Pb, Se, and Zn. A quality control sample of seawater was generated by spiking a blank seawater sample with 20 µg/L of the elements. The ICP-MS ion monitoring and resolution modes are included as Supplemental Data.

Data handling and statistical analyses

All statistical analyses were performed by applying built-in functions of the software package GraphPad Prism (Ver 8.2.1). Measured concentrations of inorganics in water and biota were first log-transformed.

The time series contained some missing data and were therefore analyzed by fitting a mixed model. This model uses a compound symmetry covariance matrix and is fit using restricted maximum likelihood. In the absence of missing values, this method gives the same *p* values and multiple comparisons tests as repeated measures analysis of variance (ANOVA). In the presence of missing values (missing completely at random), the results can be interpreted like repeated measures ANOVA. Because sphericity was not necessarily expected for all data series, a Geisser-Greenhouse correction was used.

When treatment effects were statistically significant ($p < 0.05$), Dunnett's multiple comparisons test was applied to compare data from the pebble treatment with that of the rock and slag treatments. Time series of water characteristics were analyzed the same way, with the exception that pH values were not log-transformed because these already represent a log scale.

Biomass of sponges, mussel, cockle, and bladder wrack at the end of the present study was expressed relative to the percentage of the initial biomass at introduction. After normality of these data sets was confirmed with the Shapiro-Wilk test, the significance of differences between treatments was tested with Welch's ANOVA.

GraphPad Prism was also used for preparation of all figures.

RESULTS AND DISCUSSION

Water characteristics

Water temperature, salinity, and concentrations of dissolved oxygen, DOC, inorganic nutrients (P, N, Si), and chlorophyll-a in the water column of the mesocosms were not influenced by the type of test material and remained within normal ranges for the Oosterschelde estuary throughout the present study (Table 2). Most of these parameters showed slight differences between the inflowing water and the water column in the mesocosms. This is a logical consequence of the retention time and the processes taking place in the mesocosms, like evaporation and primary production. After introduction of the pebble and

TABLE 2: Overview of water characteristics that were not affected by the test materials[a]

	Unit	Inflow	High	Low
Temperature	°C	17.3 ± 2.8	18.2 ± 3.1	18.5 ± 3.5
Salinity	‰	31.3 ± 0.9	31.7 ± 1.1	32.4 ± 1.2
Dissolved oxygen	mg/L	8.72 ± 1.60	11.36 ± 2.41	9.61 ± 1.60
Dissolved organic carbon	mg/L	2.15 ± 0.20	2.43 ± 0.26	2.72 ± 0.28
Phosphate (PO_4-P)	mg/L	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Silicate	mg/L	0.30 ± 0.01	0.30 ± 0.01	0.33 ± 0.10
Inorganic N	mg/L	0.84 ± 0.14	0.76 ± 0.11	0.76 ± 0.10
Chlorophyll-a	µg/L	0.46 ± 1.17	0.96 ± 1.30	0.82 ± 1.13

[a]Presented results are means and standard deviations of the values measured from days 0 to 84 for the refreshment water (inflow) and the water in the mesocosms with high and low refreshment rates.

rock, the turbidity increased because of the clay and silt that were associated with these test materials (Supplemental Data, Figure S1.1). Introduction of Slag did not increase turbidity.

In the mesocosms with low refreshment rates, the pH rose rapidly to 8.5 after introduction of the slag, whereas it remained stable at approximately 8.0 in the mesocosms that received rock and pebble as test material (Figure 1A). The pH increase in the slag mesocosms can be explained by the presence of calcium in the slag, which reacts with the CO_2 in the water. The nonsoluble calcium carbonate ($CaCO_3$) that was formed in this process became visible as white substrate on the outside of the slag (Supplemental Data, Figure S1.2). The pH remained significantly higher throughout the experiment in the slag-low mesocosms. The differences became smaller in the second half of the present study, when the pH values in the other mesocosms started to rise, most likely as a result of increasing primary production. In the mesocosms with high refreshment rates, the tested materials had no significant impact on the pH of the water column (Figure 1B).

Biology

No indications were found that primary production was affected by the presence of the test materials. The refreshment rate, however, had a pronounced impact because the higher flow rate resulted in higher nutrient fluxes. This was reflected by the development of the sessile periphyton and the macroalgae at the end of the study period, which were approximately 4 and 15 times higher in biomass in the mesocosm with the high refreshment rate compared to the low flow rate (Figure 2). The phytoplankton density in the inflowing water was low and hardly increased in the mesocosms, as indicated by average chlorophyll-a concentrations <1 µg/L during the present study period (Table 2).

Cockles and bladder wrack more than doubled their initial weight during the study, whereas mussels maintained their initial weight (Figure 3). Sponges lost some weight in the slag-low mesocosms and in the mesocosms with high refreshment rates. The development of the biomass of the above-mentioned groups did not differ significantly between mesocosms with different test materials.

Between 7 and 15 macroinvertebrate species per mesocosm were collected from the sediment samples, showing no indications of an effect of the treatment (Figure 4A). The number of species observed on the hard substrate/test material ranged from 3 to 13 per mesocosm. The highest numbers were found in the mesocosms with the high refreshment rate and were for all test materials alike. At the low refreshment rate, species richness was similar between pebble-low and slag-low but, on average, 2 times higher in the rock-low mesocosms (Figure 4B). Total abundance of macroinvertebrates in the soft sediment was substantially higher in the mesocosms with the higher refreshment rate, especially because of increasing numbers of amphipods of the family Corophiidae and polychaetes of the family Capitellidae (Figure 5). Overall, no statistically significant

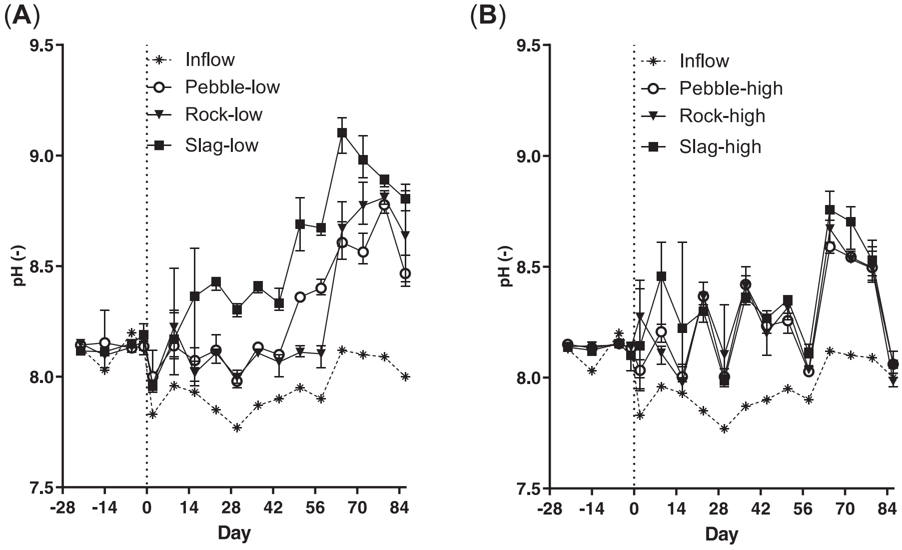

FIGURE 1: Development of pH in inflow water ($n = 1$) and in mesocosms ($n = 3$) containing pebble, rock, and slag at (**A**) low and (**B**) high refreshment rates. Mesocosm data presented as the mean (marker) and the range (error bars) of triplicates. Dotted vertical lines indicate the moment test materials were added to the mesocosms (day 0).

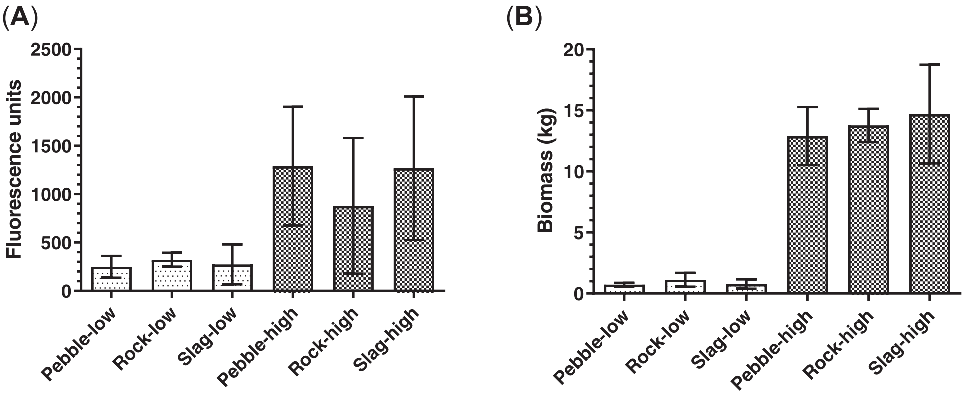

FIGURE 2: Primary producers at the end of the study of (**A**) periphyton (expressed as relative chlorophyll-a units on glass substrate) and (**B**) macroalgae (cumulative wet wt harvested during the study). Expressed as mean and range of triplicate mesocosms.

impact of the test materials on the macroinvertebrate community was detected.

Chemistry

Concentrations of inorganics in water. The concentrations of all inorganic compounds that could be measured in water are presented as figures in Supplemental Data 2. Results of the mixed-effects model analyses and the Dunnett multiple comparisons test are included as Supplemental Data, Tables S6.1 to S6.3. A selection of characteristic results is described in more detail in this section. Being no specialists in metal chemistry and to avoid a too lengthy discussion, we do not address issues like metal speciation. We are, however, open to cooperating with experts who are willing to evaluate these aspects of this data set.

Mercury, lead, selenium, tin, antimuonium, and cadmium were present only in concentrations below or just above the limit of quantification (LOQ) in the inflow and in the mesocosm water, thus without indicating that these inorganics were released from the materials. The concentrations of the other 12 compounds were detected in the inflowing water in the same range as concentrations at a reference field location (Tangelder et al. 2015; Supplemental Data, Figure S1.3). Exceptions were zinc and iron, which were present at considerably higher concentrations in the inflowing water. This suggests some contamination of the inflowing water with these compounds, probably originating from metal parts inside the pump. Zinc concentrations were lower in all mesocosms than in the inflowing water, which suggests that zinc, an essential element in many biological processes, was used by the organisms in the mesocosms.

Concentrations of arsenic, cobalt, copper, and molybdenum in the mesocosm water showed no significant relation with the test materials (Supplemental Data, Table S6.1). Arsenic and copper concentrations in the mesocosm water did not differ from the concentrations in the inflowing water. Cobalt and manganese concentrations peaked in the water of all mesocosms with low refreshment rate during the first 14 d after the introduction of the test materials. Because this peak appeared in all mesocosms, this cobalt and manganese most likely did not originate from the test materials but was released from the sediment when it was disturbed by the introduction of the test materials.

In the low refreshment mesocosms with rock, nickel concentrations in the water were significantly (Supplemental Data, Table S6.2) elevated during almost the whole test (Figure 6A) compared to the water in the mesocosms with pebble that served as "control." Concentration of aluminum (Figure 6C) and barium (Figure 6E) were only elevated at a single moment directly after introduction of the rock. During the first 14 d, vanadium concentrations (Figure 6D) were significantly higher in the rock mesocosms than in the pebble mesocosms but not

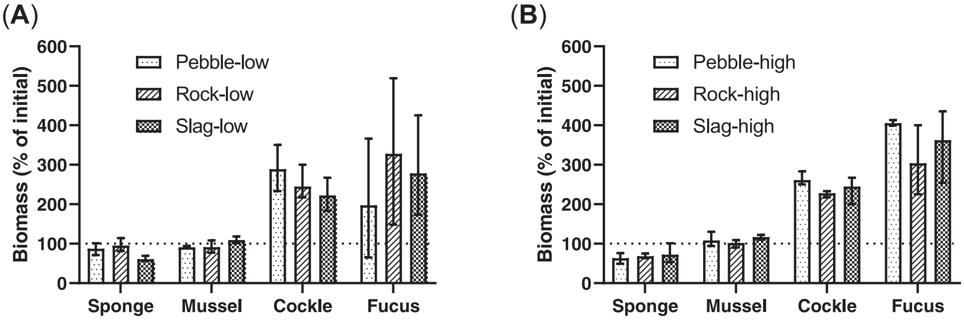

FIGURE 3: Biomass after 84 d of exposure to the test materials, expressed as percentage of the biomass at introduction of sponge, mussel, cockle, and bladder wrack (*Fucus*) in mesocosms with (**A**) low and (**B**) high water refreshment rates. Expressed as mean and range of triplicate mesocosms. The dotted line indicates the weight (100%) at the start of the study.

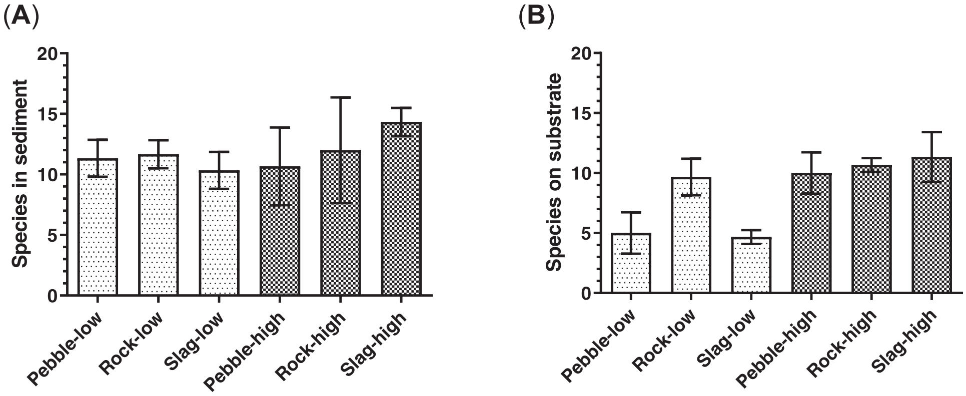

FIGURE 4: Species richness of the macroinvertebrate community on (**A**) the soft sediment and (**B**) the hard substrate/test material in the mesocosms at the end of the study. Expressed as mean and range of triplicate mesocosms.

higher than in the inflowing water. Concentrations of all other inorganics in the water of the rock mesocosms were present in the same range as in the pebble mesocosms. In the mesocosms with high refreshment rate, none of the analyzed compounds showed different concentrations between the pebble and rock treatments.

In the mesocosms with slag and low refreshment rate, the water concentrations of iron, chromium, aluminum, and vanadium peaked within the first 2 wk after the introduction of the test material. Concentrations then returned to levels similar to the pebble mesocosms. For iron, which peaked at 9 µg/L, "recovery" to approximately 3 µg/L was achieved 1 wk after the peak. Chromium reached maximum concentrations of 1.2 µg/L 2 d after the introduction of the slag. This chromium peak is evident from the graph (Figure 6B), but because concentrations in the pebble mesocosms were below the LOQ, the statistical significance could not be established. Four weeks after introduction of the slag, chromium concentrations in the water of these mesocosms were close to the LOQ (0.3 µg/L) again, similar to the pebble mesocosms.

Aluminum (Figure 6C) reached a high peak of approximately 49 µg/L 14 d after the introduction of the slag. Concentrations then gradually decreased until differences with the pebble mesocosms (3 µg/L) were no longer significant after day 42. Vanadium concentrations (Figure 6D) in the slag-low mesocosms showed a higher peak (70 µg/L) on day 14. The concentration then rapidly declined until day 21 but remained significantly higher than in the pebble mesocosms until the end of the present study. Vanadium was the only compound that also showed significantly elevated concentrations in the water column of the mesocosms with high refreshment rates (Figure 6G). Barium (Figure 6E) produced a less pronounced peak (34 µg/L) on day 21, followed by a decrease, although the concentrations remained often significantly higher than the concentrations in the water of the pebble mesocosms (17 µg/L).

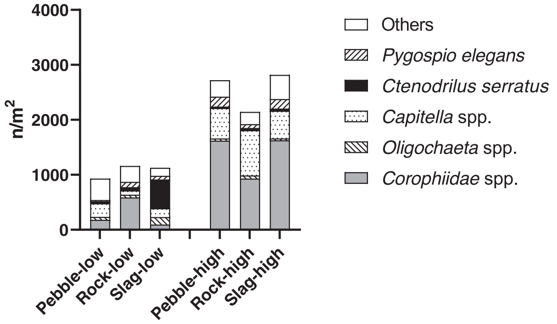

FIGURE 5: Total abundance of the most dominant macroinvertebrate species in the soft sediment of the mesocosms at the end of the study. Average values of triplicate treatments are shown.

Molybdenum concentrations were similar in all mesocosms, with the exception of the period between days 35 and 56 when a sudden decrease of the molybdenum concentration occurred in the inflowing water (Figure 6F). This decrease was reflected by decreasing concentrations in all mesocosms with high refreshment rate and in the low refreshment mesocosms with pebble and rock. In the mesocosms with slag, however, this drop was not observed. On the contrary, molybdenum concentrations slightly increased. The reason for the sudden change in the refreshment water is not known, but the data suggest that when this occurred, the slag served as a molybdenum source that in the slag mesocosms compensated for the low concentration of this element in the inflowing water.

In a leachate test with distilled water (ASTM International 2004, method D3987), BOF slag showed leaching under neutral conditions of especially aluminum, resulting in a concentration of 2.7 mg/L in the leachate water (Proctor et al. 2000). Barium (0.1 mg/L) and lead (0.03 mg/L) were also found in measurable concentrations, together with traces (all <0.01 mg/L) of arsenic, manganese, molybdenum, and vanadium. Chromium was not detectable. Strong leaching of aluminum and some of barium was also found in the marine mesocosms. However, release of vanadium and chromium that also took place in the mesocosms occurred hardly or not at all in the test with distilled water. It is unclear if this difference is due to the different exposure conditions (e.g., salinity) or indicates a different composition of the BOF slag.

Concentrations of inorganics in biota. The concentrations of all inorganic compounds that could be measured in biota are presented as figures in Supplemental Data 3 to 5. Results of the mixed-effects model analyses and the Dunnett multiple

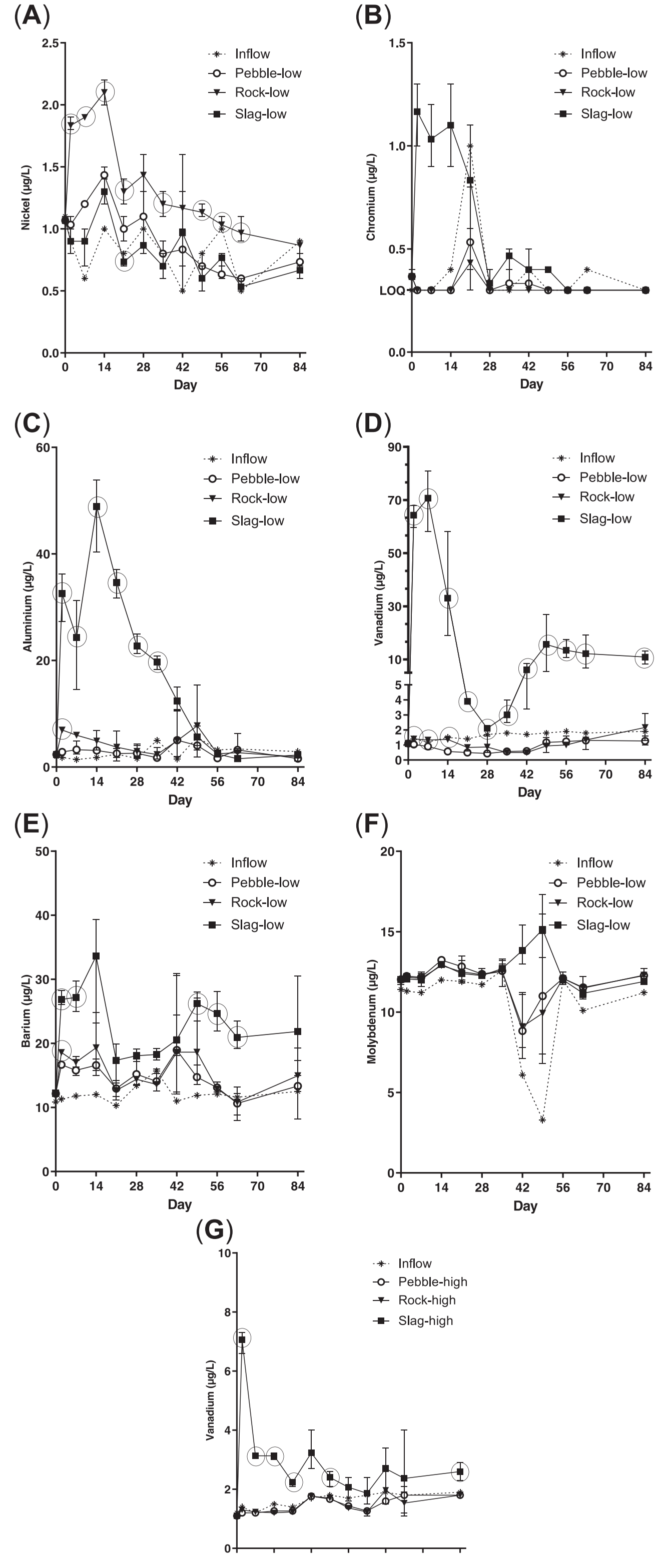

FIGURE 6: Concentrations of dissolved (**A**) nickel, (**B**) chromium, (**C**) vanadium, (**D**) aluminum, (**E**) barium, and (**F**) molybdenum in the water of the mesocosms with low refreshment rate and (**G**) vanadium in the water of the mesocosms with high refreshment rate. Mesocosm data presented as the mean (marker) and the range (error bars) of triplicates. Circled markers are significantly ($p < 0.05$) different with from the control (pebble) at that day. LOQ = limit of quantification.

comparisons test are included as Supplemental Data, Tables S6.4 to S6.9. A selection of characteristic results is described in more detail in this section.

At low refreshment rate, vanadium concentrations in the tissue of mussels (Figure 7A), bladder wrack (Figure 7C), and sponges (Figure 7D) were significantly higher in the slag mesocosms compared to the pebble mesocosms. In mussels and bladder wrack the highest vanadium concentrations were measured on day 28 at approximately 150 and 25 mg/kg dry weight. By the end of the present study (day 84) vanadium concentrations in the mussels showed a strong decline, although they were still substantially and significantly higher compared to the mussels from the pebble mesocosms. In bladder wrack some decline was also observed, but the peak levels were more or less retained until the end of the study. This difference could indicate that part of the vanadium found in the mussels was present (in the gut) as particles or that elimination of vanadium is a faster process in a mussel compared to bladder wrack. The latter could be a result of a more intense (active) water exposure of mussel tissue. Although at a much lower level, vanadium concentrations were also elevated in mussels from the slag mesocosms with high refreshment rate (Figure 7B). In these exposure conditions, elimination during the second half of the present study was not observed.

According to the European Chemicals Agency (2021), "there is no indication for biomagnification of vanadium in marine food chains." Research by Miramand et al. (1980, 1992) suggests that vanadium hardly accumulates in soft tissue of aquatic organisms but is passively concentrated on external surfaces. Using radiolabeled vanadium, they estimated that the shell contained >95% of the total vanadium load of an exposed mussel (Miramand et al. 1980). In the present study, mussel tissue was cut from the shell before chemical analyses. Because no special measures were taken to prevent the tissue from coming in contact with the outside of the shell, it cannot be excluded that some contamination of the mussel tissue occurred by touching the shell. On the other hand, it is obvious that vanadium also concentrates on soft tissue, like the gills, that has intense active contact with the water. Therefore, we do not expect that the majority of vanadium concentrations that we found in the mussel flesh were due to contamination. For bladder wrack and sponge it is (also) practically impossible to select tissue that has no direct contact with seawater for chemical analyses.

When deriving environmental risk limits in The Netherlands (Smit 2012), available bioaccumulation factors (BAFs; in liters per kilogram wet wt) for vanadium ranged from 5.5 to 27.9 for laboratory data on crustaceans and fish and from 44 to 1163 for field data on crustaceans, fish, and mollusks. The highest values were found in freshwater mussels (*Dreissena polymorpha*). From our data sets we calculated BAFs for vanadium in mussel tissue of 1039 L/kg dry weight (see Supplemental Data 7) based on the weighted average vanadium concentration in the water between days 0 and 84 and the concentration in dry weight mussel tissue on day 84. For bladder wrack, a BAF of 951 was calculated in the same way (see Supplemental Data 7).

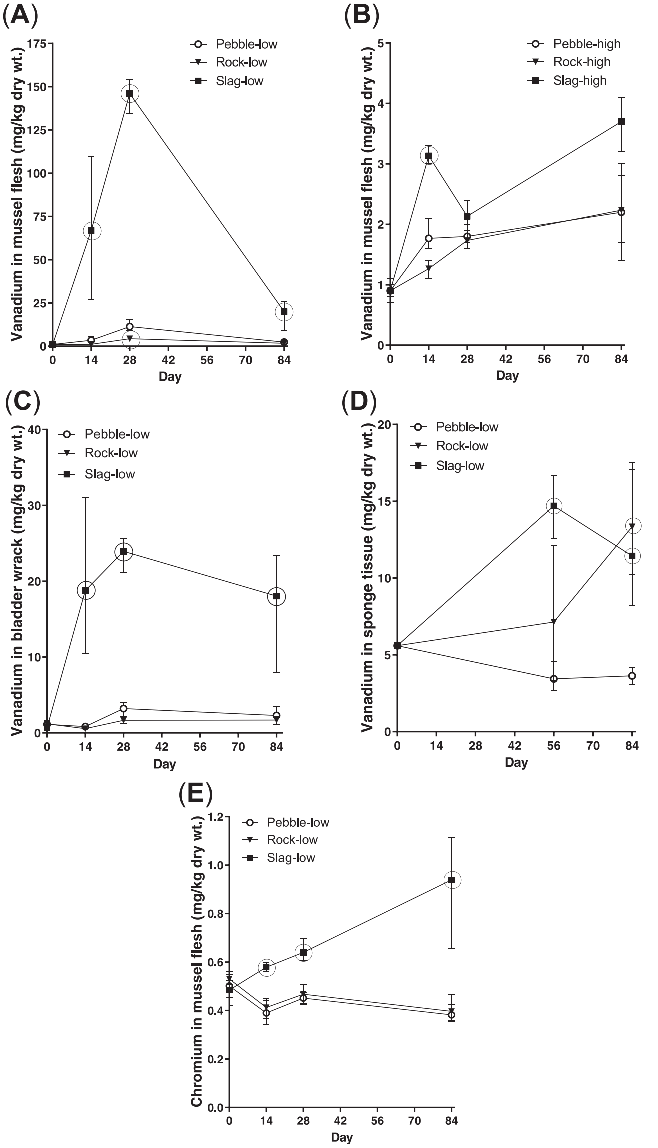

FIGURE 7: Concentrations of vanadium in mussels from mesocosms with (**A**) low and (**B**) high refreshment rates and in (**C**) bladder wrack and (**D**) sponge from mesocosms with low refreshment rate. (**E**) Chromium concentrations in mussels from mesocosms with low refreshment rate. Mesocosm data presented as the mean (marker) and the range (error bars) of triplicates. Circled markers are significantly ($p < 0.05$) different from the control (pebble) at that day.

Median effect concentration values for vanadium to marine organisms have been reported in the range of 50 to 35 000 µg/L for freshwater and marine species (Smit 2012). The lower limit of this range was exceeded in the slag-low mesocosms during the first 2 wk of the exposure period when the maximum concentration of 90 µg/L was detected, without indications of biological impact.

Besides vanadium, chromium was the only other compound that showed clearly elevated concentrations in biota. This only occurred in mussels from the mesocosms with low refreshment rate (Figure 7E). It was characterized by a steady increase until the end of the present study when chromium concentrations in the mussels from the slag mesocosms were twice as high compared to those in mussels from the pebble and rock mesocosms. It is remarkable that the chromium concentration in the mussel tissue in the slag-low mesocosms continued to increase until the end of the study, whereas chromium concentrations that were measured in the water samples reached the LOQ 30 d before the end of the study. Because the water samples were filtered prior to analysis, the majority of the chromium in the water column may not have been present in a dissolved state. It was probably absorbed by suspended particles and/or phytoplankton that were ingested by the mussels. Because water concentrations are not known, it was not possible to calculate a BAF for chromium from this data set.

The presence of the slag resulted in a pronounced elevation of the aluminum concentrations in the water of the mesocosms with low refreshment rate, but this was not reflected by increased aluminum concentrations in biota. Apparently, aluminum had a low tendency for bioaccumulation under the circumstances in the mesocosms. Bioaccumulation of aluminum in fish gills has been shown by low pH values between 5 and 6 (Teien et al. 2006). In the mesocosms, pH values were never <8, which can be regarded as a normal value for marine systems. Many metals tend to precipitate at higher pH values, but aluminum shows the lowest solubility at approximately pH 6 to 7 and has a high solubility at pH >8 (and <5; Santore et al. 2018). The pH of the seawater, which was further increased by the presence of the calcium in the slag, may therefore have facilitated the leaching of especially aluminum to the water column. The chemistry data from the mesocosms with low refreshment rate suggest that the available aluminum in the slag became depleted after approximately 60 d. The maximum aluminum concentrations in the mesocosm water were measured on day 14 at approximately 50 µg/L, which is far below the lowest no-effect concentration of 1300 µg/L, which was established at pH 8 by Gensemer et al. (2018) for a fish, an invertebrate, and an algal species. This suggests that it is unlikely that aluminum caused negative effects on the mesocosm community.

Comparing mesocosm results with field data

Results from the field monitoring in the Oosterschelde estuary between 2009 and 2014 did not show a clear link between heavy metal concentrations in water and biota and the presence of steel slag or with the diversity of the local benthic community (Tangelder et al. 2015). This is in line with the observations from the present mesocosm study with the high refreshment rate, which can be considered representative of the field situation. Even in the mesocosms with a low refreshment rate—reflecting an extreme worst-case situation with very limited water exchange—no ecological impact on the mesocosm ecosystem could be detected.

The chemical data from the field monitoring showed that mercury, cadmium, selenium, and molybdenum were sometimes present in biota, including mussels and bladder wrack, at higher concentrations than before steel slag was introduced into the environment. A link with the presence of steel slags, however, could not be established (Tangelder et al. 2015). The chemical data from the mesocosms with high refreshment rate did not show elevated concentrations in water or biota of any of these metals but only increased concentrations of vanadium in the water and mussel tissue. Even with low refreshment rates, concentrations of mercury, cadmium, selenium, and molybdenum in both water and biota were not elevated in the steel slag mesocosms. Vanadium and chromium accumulated in the tissue of mussels and bladder wrack in these mesocosms, but these metals were not found to be elevated during the field monitoring. It seems, therefore, very likely that the chemical composition of the biota that was collected during the field monitoring was not influenced by the presence of steel slags.

CONCLUSIONS

Assessing the impact of local disturbances in a field situation is often hampered by the natural and structural variation between test and reference locations, which also limits the possibility for proper replication. As a consequence, subtle effects might not be detected during field monitoring. If this is not anticipated, it could result in an underestimation of the actual impact of the disturbance. Therefore, field monitoring may not be sufficient to take away potential (public) concern.

In the mesocosm study the impact of steel slag could be tested at different exposure levels in a triplicate setup that allowed proper statistical analyses. The results indicate that no impact of the steel slag on the ecosystem is to be expected at water refreshment rates that are realistic for the Oosterschelde estuary. Even at 35 times lower refreshment rates, no ecological impact could be detected in the mesocosms.

The mesocosm further revealed that for this type of steel slag vanadium forms the best element for monitoring the chemical influence of this material in the marine environment. It is only present at very low background concentrations in water and biota but has a strong potential for accumulation in biota, maybe through strong absorption to external tissues. The fact that vanadium was not detected at higher or elevated concentrations during the field monitoring indicates that concentrations of inorganics in the sampled biota were not affected by the presence of steel slag.

The present study shows the added value of a tailor-made mesocosm study. In this case the mesocosm study was conducted after the field monitoring was performed. By conducting a mesocosm study in an earlier stage of the project, the results can also be used for designing a more tailor-made, and thus more effective, field monitoring program.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at https://doi.org/10.1002/etc.5132.

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Data Availability Statement—Data, associated metadata, and calculation tools are available from the corresponding author (edwin.foekema@wur.nl).

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