Multisite greenness exposure and oxidative stress in children. The potential mediating role of physical activity

Published version:

DOI:10.1016/j.envres.2022.112857

Environmental Research

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--Manuscript Draft--

Manuscript Number:	ER-21-1928
Article Type:	Research paper
Section/Category:	Environmental Health & Risk Assessment
Keywords:	Green spaces; oxidative status; outdoor exercise; children health; NDVI
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Abstract:	Residential greenness exposure has been reported to positively impact health mainly by reducing overweight/obesity risk, improving mental health and physical activity. Less is known on biological pathways involved in these health benefits. We aimed to investigate the association between multisite greenness exposure and oxidative stress in children and explore the potential mediating role of physical activity in this association. This cross-sectional study involved 323 healthy subjects (8-11 y) from five schools in Asti (Italy). Children's parents filled a questionnaire providing the residential address, parental education, and physical activity frequency. Oxidative stress was quantified in spot urine by isoprostane (15-F2t-IsoP) using ELISA technique. Residential and scholastic greenness were defined by the Normalized Difference Vegetation Index (NDVI) in 100, 250, 300, 500 and 1000-m buffers, and vegetated portion was also estimated. Multisite exposures were derived accounting for NDVI around home and school, weighted for time spent in each location. Linear mixed models, age-adjusted, with schools as random intercept, tested the association between 500-m multisite grenness variables and log(15-F2t-IsoP), reporting decreased oxidative stress for each unit of increase in multisite NDVI (β: -0.63, 95%CI -1.27 to 0.02) and multisite vegetated portion (β: -0.50, 95%CI -0.98 to -0.02). Adding physical activity frequency to the models slightly attenuated the magnitude of the associations for multisite NDVI (β: -0.51, 95%CI -1.16 to 0.13) and multisite vegetated portion (β: -0.42, 95%CI -0.90 to -0.07). Children reporting the lowest and the highest physical activity frequencies showed the highest levels of 15-F2t-IsoP compared to those with moderate frequency, considering both multisite NDVI and multisite vegetation portion (β: +0.23, 95%CI 0.04 to 0.43; β: +0.26, 95%CI 0.04 to 0.46; β: +0.19, 95%CI 0.04 to 0.43; β: +0.25, 95%CI 0.04 to 0.46). Multisite greenness exposure is associated with decreased oxidative stress in children and physical activity could partly mediate this relationship.
Suggested Reviewers:	Giovanna Cilluffo giovanna.cilluffo@irib.cnr.it Dr Giovanna Cilluffo is a statistician researcher who focused her research mainly on pediatric population and the influence of environmental characteristics including green spaces, air pollution, etc. She may provide a specific point of view more oriented on the health-related consequences of environmental conditions and the statistical approach we have used in our manuscript.
	Iana Markevych iana.markevych@med.uni-muenchen.de Dr Iana Markevych is a qualified research who can provide her point of view and

	precious suggestions to improve the current manuscript because (1) She recently pubblished interesting papers on environmental factors and human health; (2) She already valuably investigated the association between green spaces and human health, also focusing on the pediatric population. Therefore, she can provide insights from general and specific perspectives on this topic
	Francisco Jesus Llorente Cantarero fllorente@uco.es Dr Llorente Cantarero has a documented research activity on physical activity implications on human health. This specific expertise may help us to improve our manuscript because our secondary finding speculates on physical activity frequency and oxidative stress in children
	Eija Parmes eija.parmes@vtt.fi Dr. Parmes is a researcher in remote sensing and her contribution as reviewer could add interesting suggestion and she can help us to improve our manuscript because of her expertise on greenness exposure in children.
	Elaine Fuertes e.fuertes@imperial.ac.uk Dr. Fuertes has published many works on three different topics that we investigated in our manuscript, namely physical activity, environmental health (in children) and greenness exposure. Thus her support can be appreciable because she has an extensive expertise, documented by her research products, that can provide us a more comprehensive point of view.

Cover letter – Environmental Research

UNIVERSITY OF TURIN, ITALY
Department of Public Health and Pediatrics
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March 29th, 2021

Dear Editors of Environmental Research,

Please find attached the manuscript "*multisite greenness exposure and oxidative stress. The potential mediating role of physical activity in children*" that we would like to bring to your consideration.

In my view, the relevance of our findings can be summarised as follows.
First, the **novelty** of our findings, which add important insights in an **under-investigated topic** and use a more **comprehensive exposure characterisation** by accounting for two locations (school and residence) visited throughout the day. Second, the evidence that individual and **multisite greenness exposure is associated with systemic oxidative stress** levels in children, independently from age and other confounders. Third, the indication that the aforementioned association could be **partly mediated by physical activity** frequency. Four, the **robustness** of our findings has been confirmed by several sensitivity analysis. Finally, our data have possible future implications on Public Health and its relation with the environment by providing new evidence basis and/or serving urban planning, policy-makers decisions and general population.

Let me clarify that we accomplished all the Ethical Principles for Research involving human subjects by asking them their informed consent and the approval of the Ethic Committee " A.O.U. Luigi Gonzaga" (No. 0005540, protocol II, cat. 02, Cl. 01), in accordance with the Declaration of Helsinki.

Moreover, all of the authors have read and approved the paper and it has not been published previously nor is it being considered by any other peer-reviewed journal.

Please find below some suggestions for potential reviewers, as requested.

- Dr Giovanna Cilluffo, email: giovanna.cilluffo@irib.cnr.it;
- Dr Iana Markevych, email: iana.markevych@med.uni-muenchen.de
- Dr Llorente Cantarero, email: fllorente@uco.es

Hoping that the current manuscript may fulfil the scientific standards of Environmental Research.

Best Regards,

Prof Roberto Bono

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HIGHLIGHTS:

1. Greenness exposure is related to health but oxidative stress induction is under-investigated;
2. Multisite greenness calculations enhanced the exposure characterization throughout the day;
3. Multisite greenness is associated to lower oxidative stress, independently from confounders;
4. Physical activity may partly mediate greenness and oxidative stress association;
5. Our data may support urban green area policies for health and well-being improvement.

Multisite greenness exposure and oxidative stress. The potential mediating role of physical activity in children.

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ABSTRACT

Residential greenness exposure has been reported to positively impact health mainly by reducing overweight/obesity risk, improving mental health and physical activity. Less is known on biological pathways involved in these health benefits. We aimed to investigate the association between multisite greenness exposure and oxidative stress in children and explore the potential mediating role of physical activity in this association. This cross-sectional study involved 323 healthy subjects (8-11 y) from five schools in Asti (Italy). Children's parents filled a questionnaire providing the residential address, parental education, and physical activity frequency. Oxidative stress was quantified in spot urine by isoprostane (15-F_{2t}-IsoP) using ELISA technique. Residential and scholastic greenness were defined by the Normalized Difference Vegetation Index (NDVI) in 100, 250, 300, 500 and 1000-m buffers, and vegetated portion was also estimated. Multisite exposures were derived accounting for NDVI around home and school, weighted for time spent in each location. Linear mixed models, age-adjusted, with schools as random intercept, tested the association between 500-m multisite grenness variables and log(15-F_{2t}-IsoP), reporting decreased oxidative stress for each unit of increase in multisite NDVI (β: -0.63, 95%CI -1.27 to 0.02) and multisite vegetated portion (β: -0.50, 95%CI -0.98 to -0.02). Adding physical activity frequency to the models slightly attenuated the magnitude of the associations for multisite NDVI (β: -0.51, 95%CI -1.16 to 0.13) and multisite vegetated portion (β: -0.42, 95%CI -0.90 to -0.07). Children reporting the lowest and the highest physical activity frequencies showed the highest levels of 15-F_{2t}-IsoP compared to those with moderate frequency, considering both multisite NDVI and multisite vegetation portion (β: +0.23, 95%CI 0.04 to 0.43; β: +0.26, 95%CI 0.04 to 0.46; β: +0.19, 95%CI 0.04 to 0.43; β: +0.25, 95%CI 0.04 to 0.46). Multisite greenness exposure is associated with decreased oxidative stress in children and physical activity could partly mediate this relationship.

KEYWORDS:

Green spaces, oxidative status, outdoor exercise, children health, NDVI

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethics Committee Approval

In accordance with the principles of the Declaration of Helsinki, ethics approval (No. 0005540, protocol II, cat. 02, Cl. 01) was obtained from the A.O.U. Luigi Gonzaga Ethics Committee. Both parents or legal tutors and children signed an informed consent prior to participate.

1. Introduction

An accumulating body of evidence has shown that access and exposure to greenness are related to positive health effects, such as reduced risk of overweight and obesity, improved mental health, higher birth weight, increased physical activity, and lower mortality rates (Fong et al. 2018; James et al. 2015). For some health outcomes, such as asthma or allergic diseases, findings are inconsistent (Fuertes et al. 2016; Hartley et al. 2020; Lambert et al. 2017), which could be explained by the limited understanding of mechanisms of action of greenness. In general, greenness exposure acts on health outcomes via modification of three main factors. First, greenness has been reported to mitigate the harmful effects of other environmental risk factors, such as air and noise pollution (Dadvand et al. 2012; De Ridder et al. 2004; Wolch et al. 2014; Yang et al. 2005). Second, greenness may modify behaviour: proximity to urban vegetated areas, such as parks and gardens, and greater amount of greenness have been related to higher practice of outdoor physical activity both in children and adults (Fong et al. 2018; Gray et al. 2015; Grigsby-Toussaint et al. 2011; Markevych et al. 2016; McMorris et al. 2015), which in turn would reduce risk of diverse non-communicable diseases via body weight control. Third, greenness increases socialisation and reduces general stress, which in turn affect the risk of other diseases (Hartig et al. 2014). Surprisingly, less is known on the direct effects of greenness on biological pathways, including effects on immune system due to exposure to micro-organisms, vitamin D synthesis due to exposure to sunlight, or inflammation and oxidative stress. The latter is especially important to disentangle the mixed findings about the role of greenness on respiratory and allergic diseases. Only few observational studies have reported, in adults, an association between residential greenness and lower levels of general oxidative stress (Yeager et al. 2018) and longer telomeres, whose length represents a sort of cellular memory of oxidative stress and inflammation episodes (Martens and Nawrot 2018; Woo et al. 2009). To our knowledge, no data is available on the potential effects of greenness on oxidative stress in children, except for a recently published article that, however, did not address the possible confounding covariates (De Petris et al. 2021).

Therefore, we aimed to assess the association between outdoor multisite greenness exposure and oxidative stress in children, and to explore the potential mediating role of physical activity in the mentioned association.

2. Materials and methods

2.1. Study design, setting and participants

This cross-sectional study involved 323 healthy children aged from 8 to 11 years, recruited as consecutive sample between March and May 2017 from five primary schools of Asti, a small town located in Piedmont, north-western Italy. In accordance with the principles of the Declaration of Helsinki, ethics approval (No. 0005540, protocol II, cat. 02, Cl. 01) was obtained from the A.O.U. Luigi Gonzaga Ethics Committee. Both parents or legal tutors and children signed an informed consent prior to participate.

2.2. Children characteristics

Demographic characteristics of the subjects, including date of birth, sex, residential address, parental education and physical activity frequency were assessed using a standardised questionnaire, "SIDRIA" (Galassi et al. 2005). Parental education levels were assessed separately for mothers and fathers and categorised as *Elementary school, Low secondary school, High secondary school, and University degree or beyond*. Physical activity frequency was assessed by asking the question "*How many days per week does your child play sport or spend time being active/doing physical activity for at least 60 minutes?*" The answer (days) underwent re-codification into 4 groups: ≤1 day/2/3/≥ 4 days. Anthropometric measurements (weight and height) were done by a skilled operator, during morning school hours, using a scale (FitScan BC-545F Tanita®) and a stadiometer (GIMA professional medical products). All children wore light clothes, without shoes and socks during this phase. Body mass index (BMI) was calculated dividing body weight (Kg) by squared height (m^2). A spot of fresh urine was provided by all participants during the morning hours of weekdays and maintained at +4°C during the commuting between schools and laboratory, where samples were aliquoted and stored at −80 °C until analyses, performed within 2 months. Cotinine was quantified in urinary samples to objectively assess the exposure to tobacco smoke by performing a double liquid-

liquid extraction followed by a GC-MS determination, as described elsewhere (Bono et al. 2016). To normalise the excretion rate of both urinary biomarkers, urinary creatinine (crea) was quantified by the kinetic Jaffé procedure (Bonsnes and Taussky 1945) and the biomarker quantifications were expressed as ng/mg crea.

2.3. Outdoor greenness exposure

We assessed greenness using the Normalised Difference Vegetation Index (NDVI). NDVI is a widely used index of vegetated biomass that ranges from –1 to 1 and is calculated from the leaves chlorophyll that mostly reflects the near-infrared band (NIR) (0.7–1.1 µm) and strongly absorbs the visible light (0.4–0.7 µm). A multispectral and cloud-free image (resolution 10 x 10 m), acquired on 06 July 2017 by Sentinel-2 (S2) satellite, was downloaded from Theia CNSE website (https://www.Theia-land.fr/en/product/sentinel-2-surface-reflectance/) and used for the greenness calculations, assuming that June-July months are the period of maximum vegetation phenology expression in the study area (De Petris et al. 2019; Zhou et al. 2016). For each participant we derived NDVI within 100 m, 250 m, 300 m, 500 m and 1000 m buffers surrounding the geocoded residential and school address. We also derived the vegetated portion as the ratio between the vegetated area (calculated by masking out non-vegetated pixels, i.e. those with NDVI < 0.40) and the whole buffer area. For each participant we derived two multisite greenness measures (i.e. multisite NDVI and multisite vegetated portion) by averaging residential and school greenness variables weighted by the time that children spend at school (8h/day) and at home (16h/day).

2.4. Oxidative stress

Oxidative stress was assessed by the urinary levels of isoprostane. Urinary isoprostane (15-F_{2t}-IsoP) was quantified as systemic biomarker of oxidative stress by ELISA technique, according to manufacturer's instructions (Oxford, MI, USA). The β-glucuronidase was added to each urinary sample and incubated for two hours at 37 °C. This preliminary procedure allowed to detect the total quantity of 15-F_{2t}-IsoP, which is mostly excreted as glucuronic acid conjugated (over 50%). A dilution 1:4 was performed to improve the assay accuracy (Romanazzi et al. 2013). Intra-assay variability was 9.3% (plate average CV %) and inter-assay variability was 9.3-13.3% (inter plates average CV %).

2.5. Statistical analysis

Based on previous publications on NDVI distribution in the same geographic area (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6981614) and urinary isoprostane levels distribution in a similar population (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6604009/), the available 323 participants allow to detect a reduction ≥20% in isoprostane per 1 unit of NDVI with a statistical power of 77%. Due to the small proportion of missing data (< 3.5% for parental education variable only), we used a complete case strategy and reported missing data in the table footnotes, were needed. Continuous variables are presented as mean (Standard Deviation, SD) or, if the distribution was not normal, median (Interquartile Range, IQR). Categorical variables are expressed as absolute and/or relative frequency (number of cases; %). Since sex-based differences in oxidative stress have been extensively reported in previous literature in both adults (Kander et al. 2017) and children (Llorente-Cantarero et al. 2013), differences between boys and girls were tested using non-parametric Mann-Whitney U-test with continuous variables (age, BMI, oxidative stress, greenness variables) and X^2 test with categorical variables (sampling location, physical activity and parental education). Shapiro-Wilk test and QQ-plots were used to assess normality, thus the oxidative stress measure (15-F_{2t}-IsoP) was log-transformed (log-e) to achieve a normal distribution. We defined multi-site NDVI and multisite vegetated portion within 500 m buffer as the main exposure variables. The association between multisite greenness variables and oxidative stress (log 15-F_{2t}-IsoP) was assessed using a Linear Mixed Model, one at a time, including schools as a random intercept, to account for the potential heterogeneity due to sampling. The parsimonious model was age-adjusted and was fitted by the Restricted Maximum Likelihood REML estimation. The goodness of fit was checked by verifying the normality of the residuals. To explore the potential mediation role of physical activity in the association between greenness and oxidative stress we added it as a covariate to the models (Baron and Kenny 1986). In exploratory descriptive analyses the association between physical activity and isoprostane showed a U shape, thus the group of 2 days/week of physical activity was chosen as the reference category. As sensitivity analyses, (1) we further adjusted for BMI, sex, cotinine level, and parental education, to reduce potential residual confounding in the main analysis, as some of these covariates have been reported associated with exposure and outcome in the literature; (2) we repeated the main analysis using different buffer sizes (100 m, 250 m, 300 m and 1000 m buffer, one at a time) to reduce potential measurement error in the exposure variables; and (3) we estimated the association between greenness variables and oxidative stress by schools and combined those estimates using the meta-analytical method of the inverse variance, DerSimonian-Laird estimator for Tau^2 and Jackson method for confidence intervals, to account for potential model miss-specification. The statistical significance was set at $\alpha = 0.05$ with a confidence interval CI = 95%. All statistical analyses were performed using SPSS software (IBM SPSS® Statistics version 26) and R (version 3.6.2).

3. Results

3.1. Subjects characteristics

Overall, 323 children were included in the analyses. Table 1 shows the demographic characteristics of the population sample. Mean age was 9.1 ± 1.0 (years) and 50% of them were females. According to their BMI, over a third of the participants was overweight or obese (41%). Only 6.2% of subjects was living in the countryside neighbouring the town of Asti. The majority of the participant homes and the five primary schools where placed within the Asti boundaries, in the urbanised area. Almost 53% of children were moderately active, reporting a frequency of episodes of at least 60 min of physical activity on 2-3 days per week. Over half of the parents had a high school diploma or a university degree or higher (56%). Around 3% of mothers and the same percentage of fathers did not report the education level. Girls were more exposed than boys to passive tobacco smoke ($p = 0.021$) but no difference by sex was observed for age, BMI, 15-F_{2t}-IsoP, greenness variables or parental education. Multisite greenness exposures were different ($p < 0.001$) among children attending different schools, ranging from 0.11, in children attending school 4 and 5, to 0.96, in children attending school 4 and from 0.13 in children attending school 4 and 5 to 0.81 in those attending school 2 (Figure 1).

3.2. Association between greenness and oxidative stress

In the multivariable models, all greenness variables were inversely associated with isoprostane levels, attesting decreased oxidative stress for each unit of increase in multisite NDVI (β: -0.63, 95%CI -1.27 to 0.02) and multisite vegetated portion (β: -0.50, 95%CI -0.98 to -0.02), but the association was statistically significant only for multisite vegetated portion (Table 2). The addition of physical activity frequency to the models slightly attenuated the magnitude of the associations between greenness variables and oxidative stress. Being in the lowest and highest frequency of physical activity was associated with higher levels of oxidative stress (β: +0.23, 95%CI 0.04 to 0.43; β: +0.26, 95%CI 0.04 to 0.46; β: +0.19, 95%CI 0.04 to 0.43; β: +0.25, 95%CI 0.04 to 0.46) compared to those with moderate frequency (Table 2).

3.3. Sensitivity analyses

Sensitivity analyses adjusting for additional covariates and considered different buffers of greenness exposure provided similar associations between greenness and oxidative stress, with a trend toward stronger associations as the buffer size increases (Table 3, Figure 2, and supplementary Table S1). Further, the results of meta-analysis showed that there was no evidence of heterogeneity across schools (Figure 3).

4. Discussion

This study reports for the first time the association between multisite greenness exposure and oxidative stress in children. Main results are that: (1) higher greenness exposure relates consistently to lower oxidative stress levels; and (2) the observed associations are attenuated when physical activity is included in the model. Potential confounding by subject characteristics, heterogeneity by area, or exposure misclassification do not seem to explain the results. The observed association between greenness and oxidative stress in children is in agreement with a previous report in a small cross-sectional study of adults from Kentucky, USA, in which residential NDVI (n = 82, adults) was inversely associated with oxidative stress, after adjustment for multiple potential confounders (Yeager et al. 2018). Noticeably, only the analyses including 500 m and 1,000 m radii reached the statistical significance in consistence with recent literature, which reported that buffers between 500 and 999 m in size best predict greenness effect on physical health, while buffers smaller than 500 m or greater than 999 m, are less predictive (Browning and Lee 2017). Some biological mechanisms could explain a direct effect of greenness exposure on oxidative stress. First, higher greenness exposure affects the immune-system regulation. In fact, it has been reported that the massive increase in inflammatory disorders in high-income countries is partly related to scarce or inadequate exposure to some categories of organisms that normally colonise natural environments and are able to drive the immunoregulatory mechanisms in humans (Rook 2013) or contribute to the immune system development in children (Aerts et al. 2018). Second, higher exposure to greenness may increase the synthesis of Vitamin D, provided that urban green spaces are effectively used by city dwellers. In this case, children would be more exposed to sunlight, whose ultraviolet B rays' component directly stimulates the synthesis of Vitamin D. This latter is known as one of the physiological anti-oxidants that prevents from oxidative stress-related detrimental effects and from inflammation (Wimalawansa 2019) also in youth (Filgueiras et al. 2020). It has also been suggested that greenness exposure may exert an indirect effect on oxidative stress via physical activity. In a recent literature review (Fong et al. 2018) increased greenness or proximity to green spaces have been associated to higher participation in physical activity, which in turn affects oxidative stress (Powers et al. 2020). This hypothesis is supported by our finding that the inclusion of physical activity as a covariate in the model attenuated the association between greenness and oxidative stress. This is in agreement with previous literature investigating the relationship between greenness and physical activity, both in youths and adults (James et al. 2015). In a cohort study of 365 pre-schoolers from Illinois, increased physical activity was associated with higher NDVI (Grigsby-Toussaint et al. 2011). Similar results were found in 3042 adolescents from Wesel (Markevych et al. 2016) and in 69,910 young Canadian adults (McMorris et al. 2015). We observed that physical activity frequency is non-linearly related to oxidative stress levels. Specifically, we found that children reporting the highest physical activity frequency (≥ 4 days/week at least 60 minutes) and those engaged in the lowest physical activity frequency (0-1 days/week at least 60 minutes) showed the highest levels of oxidative stress compared to children who exercised up to 2 days per week, 60 minutes each. This is in agreement with previous data reporting that too high physical activity levels are linked to chronic oxidative insults, while low frequency lacks in stimulating the anti-oxidant defence systems, and only mild frequency is able to stimulate the repair pathways counteracting oxidative stress in young adults (Pittaluga et al. 2006). Our findings might have several implications (i) for future research, since they provide new piece of evidence on a mechanism that has not yet explored, which opens new hypothesis and provides supporting data on this topic; (ii) for public health interventions, because they support urban green spaces policies and reinforce the awareness on the contribution that physical activity and lifestyles play in health and well-being, especially in susceptible populations as children and (iii) for the general population, as they advise children to spend more time outdoors in natural environment. Future research is needed to define the mechanisms behind this association, possibly considering a longitudinal design, which allows to infer on the long-term effects of greenness exposure, on the causal relationship with oxidative stress, and on the mediating role of physical activity.

4.1. Strengths and limitations

Our study has several strengths. First, this is the first attempt to estimate the association between greenness and oxidative stress in children. Second, multisite exposures were considered weighting the exposure variables to time spent at school (8h) and time spent at home (16h) accounting for the partial movement throughout the day, thus reducing exposure misclassification. Third, we evaluated different buffers and NDVI calculations, to avoid leaving out the optimal distance of impact on oxidative stress, which has not been established yet in children, and to better detect the vegetation cover. Fourth, we included children from different schools and found no evidence of heterogeneity, indirectly supporting absence of residual confounding. Finally, to measure oxidative stress we quantified the 15-F_{2t}-IsoP, which is a reliable and acknowledged biomarker of oxidative stress quantification *in vivo* (Musiek et al. 2005). As main weaknesses, the cross-sectional design of the study did not permit to conclude on causal relationship between greenness and oxidative stress, however it is unlikely that oxidative stress status (unknown to most subjects) will prompt a change in home or school address. Potential selection bias may deserve consideration, as the participants were enrolled as volunteers. However, it is worth mentioning that all education levels were represented and all participants were healthy, as inclusion criteria. A set of covariates were considered and included in the analyses nonetheless residual confounding is still possible due to potentially unmeasured confounders known to be related to oxidative stress (e.g. exposure to air pollution). Finally, physical activity frequency and parental education, were assessed by questionnaire, which may lead to misclassification or recall bias, although a standardised questionnaire has been used.

5. Conclusions

This cross-sectional study found that multisite greenness exposure is associated with decreased oxidative stress in children and that physical activity could partly mediate this relationship. The potential short and long-term effects of such excess oxidative stress in health are unknown, deserve further investigation, and support city and public health strategies to green the environment.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We acknowledge support from the Spanish Ministry of Science and Innovation through the "Centro de Excelencia Severo Ochoa 2019-2023" Program (CEX2018-000806-S), and support from the Generalitat de Catalunya through the CERCA Program. We further thank the PhD Program in Pharmaceutical and Biomolecular Sciences of the University of Turin that supported GS's predoctoral fellow and research stay at ISGlobal in Barcelona.

Authors' contributions

Conceptualisation: G.S., R.B.; methodology: G.S., V.B., A.E.C.; validation: R.B., J.G.A.; formal analysis: G.S., A.E.C.; investigation, V.B., G.S., R.B., resources: R.B.; data curation, G.S., A.E.C., V.B., writing and original draft preparation, G.S. A.E.C., review, editing, and supervision: R.B., J.G.A. All authors (I) have read and agreed the work is ready for submission and (II) accept responsibility for the manuscript's contents.

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Characteristic	Total	Female	Male	P-value
n	323 (100%)	161 (50%)	162 (50%)	
Age (years)	9.1 ± 1.0	9.1 ± 0.9	9.1 ± 1.0	0.99
BMI (kg/m^2)	19.0 ± 3.5	19.1 ± 3.6	18.8 ± 3.5	0.27
15-F_{2t}-IsoP (ng/mg crea)	2.52 (0.20)	2.59 (0.34)	2.46 (0.19)	0.56
Cotinine (ng/mg crea)	1.22 (0.59)	1.38 (0.88)	1.08 (0.79)	0.02
Multisite NDVI 500 m buffer	0.32 ± 0.12	0.33 ± 0.12	0.32 ± 0.12	0.40
Multisite vegetated portion 500 m buffer	0.36 ± 0.16	0.37 ± 0.16	0.35 ± 0.16	0.19
Sampling location:				
School 1	99 (31)	49 (31)	50 (31)	
School 2	119 (37)	49 (30)	70 (43)	
School 3	48 (15)	26 (16)	22 (14)	
School 4	30 (9)	19 (12)	11 (7)	
School 5	27 (8)	18 (11)	9 (5)	
Physical Activity (days/week practising at least 60')				
0-1	88 (27)	51 (32)	37 (23)	
2	102 (32)	55 (34)	47 (29)	
3	69 (21)	27 (17)	42 (26)	
≥ 4	64 (20)	28 (17)	36 (22)	
Parental education				
Mother education: Upper secondary and beyond	180 (56)	90 (56)	90 (56)	>0.99
Father education: Upper secondary and beyond	162 (50)	83 (52)	79 (49)	0.75

Table 1. Demographic characteristics of the study participants by sex (n = 323)

Note: P-values are reported for chi-squared test for categorical variables and Mann-Whitney U test for continuous variables. Continuous data are presented as mean ± Standard Deviation, 15-F_{2t}-IsoP and cotinine as Geometric Mean (Standard Error). Number of subjects (frequency) are reported for categorical variables. BMI = Body Mass Index, 15-F_{2t}-IsoP = isoprostane oxidative stress biomarker, NDVI = Normalized Difference Vegetation Index and vegetated portion refers to 500-m buffer and range from -1 to -1 and from 0 to 1, respectively. Higher values indicate more greenness. Number of observations for parental education differs from the total number of observations due to missing values (n = 312).

	Mixed linear model, age-adjusted		Additional adjustment by physical activity	
	Coeff. (95% C.I.)	P-value	Coeff. (95% C.I.)	P-value
Multisite NDVI	-0.63 (-1.27, 0.02)	0.06	-0.51 (-1.16, 0.13)	0.12
Physical activity(days/week at least 60min)				
0-1			0.23 (0.04, 0.43)	0.02
2			Ref.	
3			0.16 (-0.05, 0.37)	0.12
≥4			0.25 (0.04, 0.46)	0.02
Multisite vegetated portion	-0.50 (-0.98, -0.02)	0.04	-0.42 (-0.90, 0.07)	0.09
Physical activity(days/week at least 60min)				
0-1			0.19 (0.04, 0.43)	0.02
2			Ref.	
3			0.15 (-0.05, 0.37)	0.12
≥4			0.25 (0.04, 0.46)	0.02

Table 2. Multivariable association between multisite greenness exposure and oxidative stress (log-transformed urinary 15-F_{2t}-IsoP levels)

Note: All estimates are derived from Linear Mixed Models (n = 323), with school as random intercept, age-adjusted., NDVI = Normalized Difference Vegetation Index and vegetated portion refer to 500-m buffer and range from -1 to -1 and from 0 to 1, respectively. Higher values indicate more greenness. Multisite greenness exposures are weighted by time spent at school (8h/day) and at home (16h/day).

	Fully adjusted model		Radius 100 m		Radius 250 m		Radius 300 m		Radius 1,000 m	
	Coeff. (95%CI)	p	Coeff. (95%CI)	p	Coeff. (95%CI)	p	Coeff. (95%CI)	p	Coeff. (95%CI)	p
Multisite NDVI	-0.72 (-1.39, -0.05)	0.04	-0.27 (-0.90, 0.37)	0.41	-0.47 (-1.11, 0.17)	0.15	-0.56 (-1.18, 0.10)	0.09	-0.80 (-1.50, -0.09)	0.03
Multisite vegetated portion	-0.57(-1.06, -0.07)	0.03	-0.21 (-0.64, 0.23)	0.35	-0.38 (-0.83, 0.09)	0.10	-0.43 (-0.90, 0.03)	0.07	-0.62 (-1.17, -0.07)	0.03

Table 3. Sensitivity analyses. Multivariable association between multisite greenness exposures and oxidative stress (log-transformed urinary 15-F_{2t}-IsoP levels) after additional adjustment by covariates and by buffer size

Note: All estimates are derived from Linear Mixed Models (n = 323), age-adjusted, with school as random intercept. NDVI = Normalized Difference Vegetation Index and vegetated portion refers to 500-m buffer and range from -1 to -1 and from 0 to 1, respectively. Higher values indicate more greenness. Multisite greenness exposures are weighted by time spent at school (8h/day) and at home (16h/day). Fully adjusted models include age, sex, BMI, cotinine levels, parental education. The number of observations of parental education differs from the total number of cases due to parental education missing values, thus the fully adjusted model includes 312 observations out of 323.

Figure 1. Multisite greenness exposure and oxidative stress (log-transformed urinary 15-F2t-IsoP levels) according to schools.

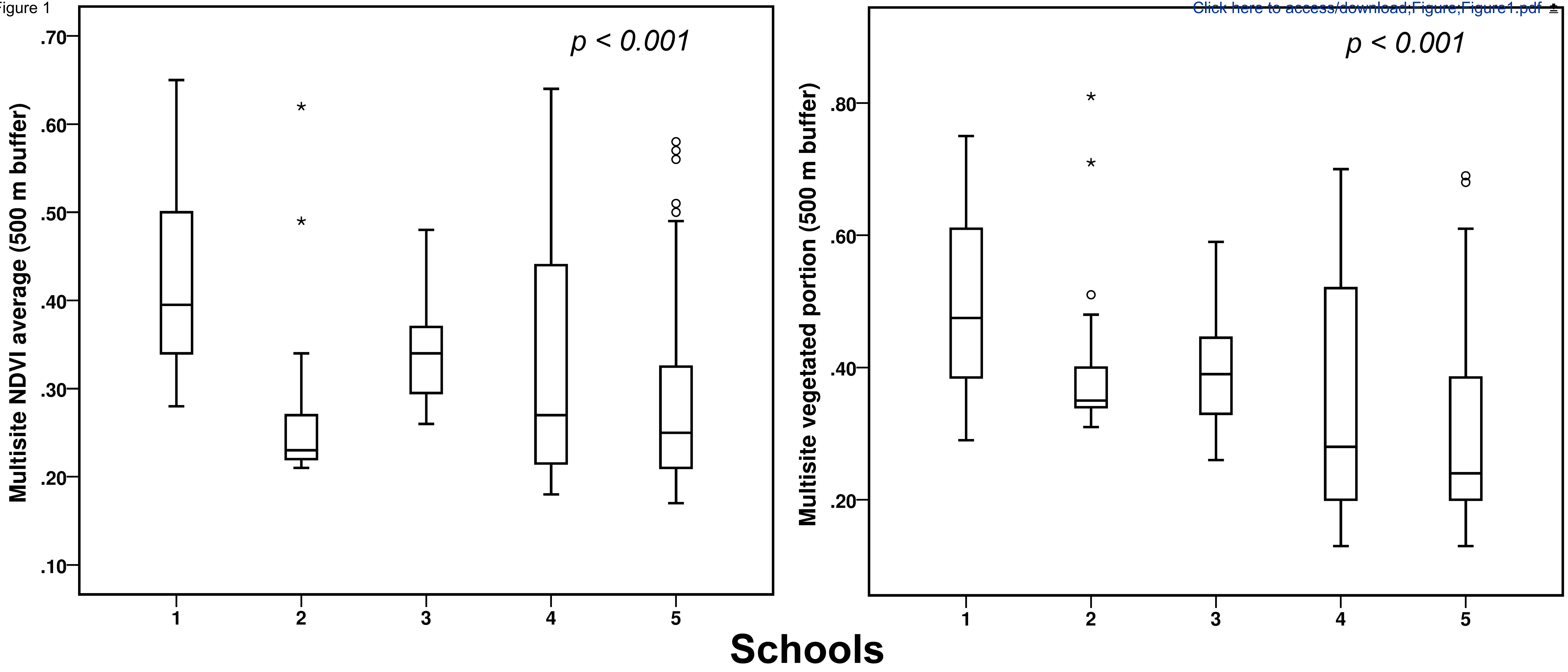
Note: P-values were derived from Kruskal-Wallis test. NDVI = Normalized Difference Vegetation Index and vegetated portion refers to 500-m buffer and range from -1 to -1 and from 0 to 1, respectively. Higher values indicate more greenness.

Figure 2. Estimate trends of the associations between multisite greenness exposure and oxidative stress (log-transformed urinary 15-F2t-IsoP levels) according to radius size

Figure 3. Forest plot of meta-analysis by school. Associations between multisite greenness exposure and oxidative stress (log-transformed urinary 15-F2t-IsoP levels) by schools

Note: meta-analysis by schools was performed by the meta-analytical method of the inverse variance, DerSimonian-Laird estimator for Tau2 and Jackson method for confidence intervals. NDVI = Normalized Difference Vegetation Index and vegetated portion refers to 500-m buffer and range from -1 to -1 and from 0 to 1, respectively. Higher values indicate more greenness.

Figure 1



p < 0.001
Multisite NDVI average (500 m buffer)
.70
.60
.50
.40
.30
.20
.10
1
2
3
4
5
p < 0.001
Multisite vegetated portion (500 m buffer)
.80
.60
.40
.20
1
2
3
4
5
Schools

Figure 2

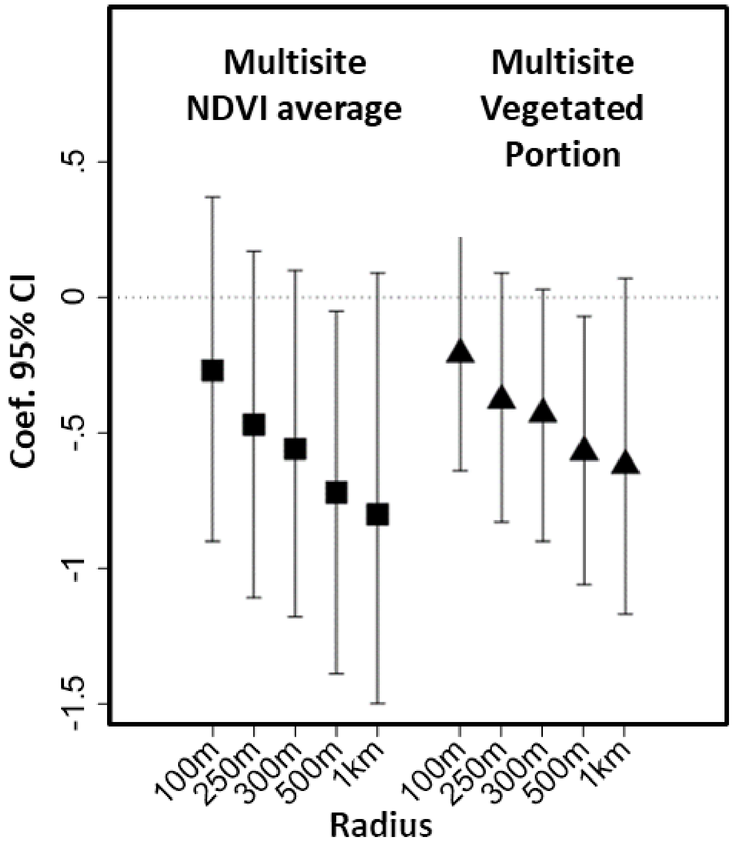
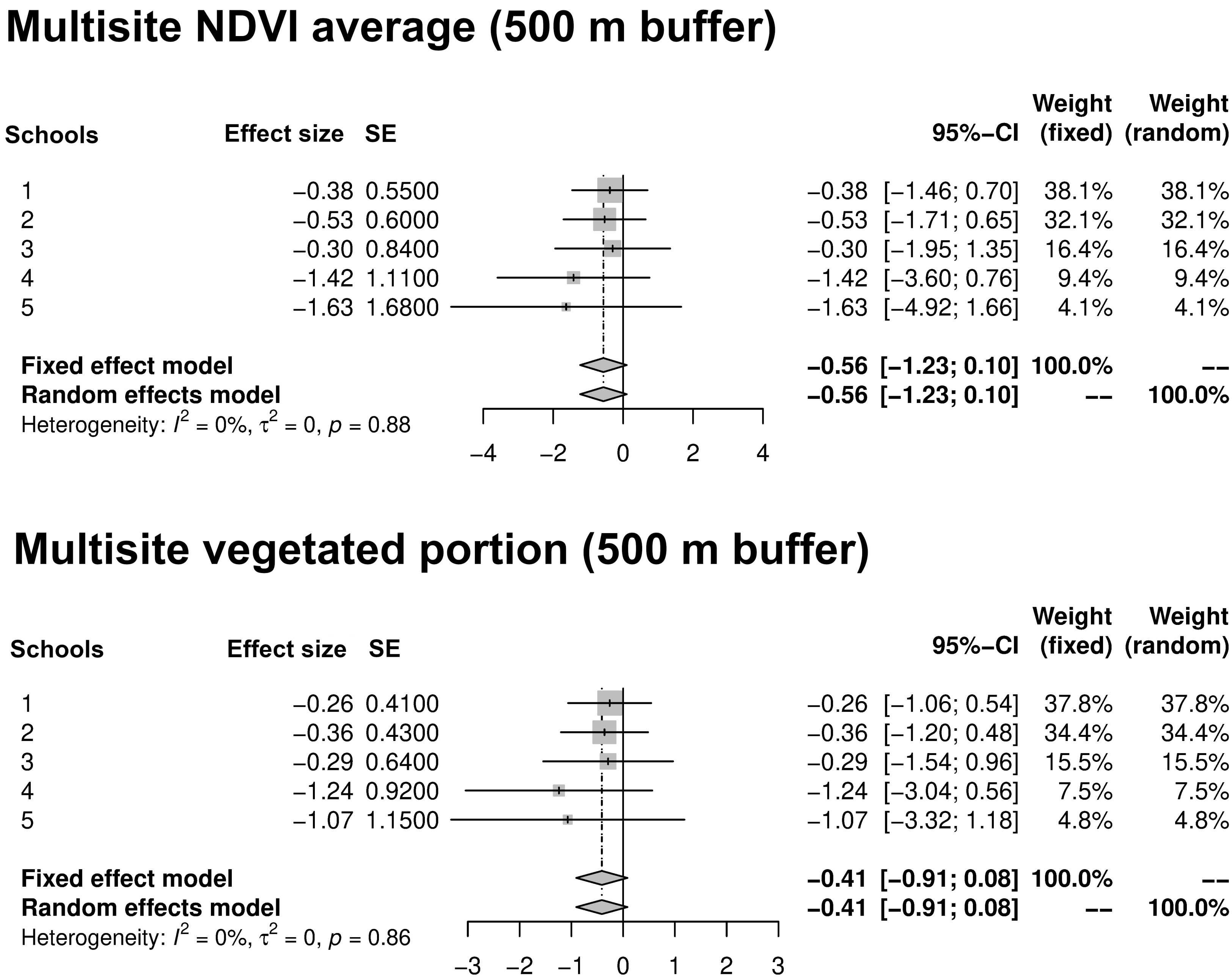

Multisite
NDVI average
Multisite
Vegetated
Portion
Coef. 95% CI
.5
0
-.5
-1
-1.5
100m
250m
300m
500m
1km
100m
250m
300m
500m
1km
Radius

Figure 3


Multisite NDVI average (500 m buffer)
Schools
Effect size
SE
95%–CI
Weight (fixed)
Weight (random)
1 −0.38 0.5500 −0.38 [−1.46; 0.70] 38.1% 38.1%
2 −0.53 0.6000 −0.53 [−1.71; 0.65] 32.1% 32.1%
3 −0.30 0.8400 −0.30 [−1.95; 1.35] 16.4% 16.4%
4 −1.42 1.1100 −1.42 [−3.60; 0.76] 9.4% 9.4%
5 −1.63 1.6800 −1.63 [−4.92; 1.66] 4.1% 4.1%
Fixed effect model −0.56 [−1.23; 0.10] 100.0% --
Random effects model −0.56 [−1.23; 0.10] -- 100.0%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.88
−4 −2 0 2 4
Multisite vegetated portion (500 m buffer)
Schools
Effect size
SE
95%–CI
Weight (fixed)
Weight (random)
1 −0.26 0.4100 −0.26 [−1.06; 0.54] 37.8% 37.8%
2 −0.36 0.4300 −0.36 [−1.20; 0.48] 34.4% 34.4%
3 −0.29 0.6400 −0.29 [−1.54; 0.96] 15.5% 15.5%
4 −1.24 0.9200 −1.24 [−3.04; 0.56] 7.5% 7.5%
5 −1.07 1.1500 −1.07 [−3.32; 1.18] 4.8% 4.8%
Fixed effect model −0.41 [−0.91; 0.08] 100.0% --
Random effects model −0.41 [−0.91; 0.08] -- 100.0%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.86
−3 −2 −1 0 1 2 3

Conflict of interests

The authors declare no conflict of interests.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: