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RESEARCH ARTICLE

Occupational exposure to diesel engine exhaust and serum levels of microRNAs in a cross-sectional molecular epidemiology study in China

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Abstract

Diesel engine exhaust (DEE) is an established lung carcinogen, but the biological mechanisms of diesel-induced lung carcinogenesis are not well understood. MicroRNAs (miRNAs) are small noncoding RNAs that play a potentially important role in regulating gene expression related to lung cancer. We conducted a cross-sectional molecular epidemiology study to evaluate whether serum levels of miRNAs are altered in healthy workers occupationally exposed to DEE compared to unexposed controls. We conducted a two-stage study, first measuring 405 miRNAs in a pilot study of six DEE-exposed workers exposed and six controls. In the second stage, 44 selected miRNAs were measured using the Fireplex circulating miRNA assay that profiles miRNAs directly from biofluids of 45 workers exposed to a range of DEE (Elemental Carbon (EC), median, range: 47.7, 6.1–79.7 μg/m^3) and 46 controls. The relationship between exposure to DEE and EC with miRNA levels was analyzed using linear regression adjusted for potential confounders. Serum levels of four miRNAs were significantly lower (miR-191-5p, miR-93-5p, miR-423-3p, miR-122-5p) and one miRNA was significantly higher (miR-92a-3p) in DEE exposed workers compared to controls. Of these miRNAs, miR-191-5p (p_{trend} = .001, FDR = 0.04) and miR-93-5p (p_{trend} = .009, FDR = 0.18) showed evidence of an inverse exposure–response with increasing EC levels. Our findings suggest that occupational exposure to DEE may affect circulating miRNAs implicated in biological processes related to carcinogenesis, including immune function.

Abbreviations: DEE, diesel engine exhaust; miRNA, microRNA; EC, elemental carbon; OC, organic carbon; RT-PCR, reverse transcription polymerase chain reaction; FDR, false discovery rate; BMI, body mass index.

Wei Hu, Jason Y. Y. Wong, and Yufei Dai contributed equally to first authorship.

Roel C. Vermeulen, Debra T. Silverman, Yuxin Zheng, Qing Lan, and Nathaniel Rothman co-supervised this work.

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1 | INTRODUCTION

Diesel engine exhaust (DEE) contains a mixture of gases and particulate matter (PM), including several substances that are classified as human carcinogens by the International Agency for Research on Cancer (IARC) including nitro-polycyclic aromatic hydrocarbons (McDonald et al., 2011). DEE has been classified as a Group 1 carcinogen by the IARC based on sufficient evidence of causing lung cancer in humans, and a positive association with bladder cancer was also noted by the IARC Working Group in 2012 (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2014). Though IARC concludes strong evidence that DEE can induce lung cancer in humans through genotoxic mechanisms including DNA damage, gene and chromosomal mutation, changes in relevant gene expression, the production of reactive oxygen species, and inflammatory responses, there remain questions as to whether DEE causes cancer through other mechanisms.

MicroRNAs (miRNAs) are a family of small noncoding regulatory RNAs consisting of about 20 nucleotides that exert regulatory functions of inhibiting messenger RNA (mRNA) translation and promoting mRNA degradation through base pairing with complementary sequences of target mRNAs (Hudder & Novak, 2008; Wu et al., 2019). miRNAs play important roles in biological functions including developmental timings, cell differentiation, embryogenesis, metabolism, organogenesis and apoptosis, and cell–cell communication (Mohr & Mott, 2015; Saliminejad et al., 2019; Vidigal & Ventura, 2015). They can be altered by exposure to metals, cigarette smoke, persistent organic pollutants, and air pollution (Hou et al., 2011; Krauskopf et al., 2017, 2018; Vrijens et al., 2015). However, findings on the associations between constituents of air pollution and miRNA were inconsistent and there was little overlap among in vitro, in vivo, and human studies (Krauskopf et al., 2019; Vrijens et al., 2015).

Several environmental, human bronchial epithelial cells, and crossover studies have assessed the association between diesel exhaust and altered expression of miRNAs (Cheng et al., 2020; Jardim et al., 2009; Krauskopf et al., 2018, 2019; Yamamoto et al., 2013). However, few studies to date have investigated the potential exposure-response relationship between DEE exposure and the miRNAs expression alteration.

To evaluate the relationship between long-term, occupational DEE exposure and circulating miRNAs, we conducted a cross-sectional molecular epidemiology study of diesel engine testing facility workers and comparable controls. The findings from our study could potentially provide insight into the molecular mechanism of DEE-related carcinogenesis.

2 | MATERIALS AND METHODS

2.1 | Study design and exposure assessment

Characteristics of the overall population and study design have been described elsewhere (Lan et al., 2015). Briefly, the study population included 54 male workers exposed to DEE in a diesel engine manufacturing facility and 55 male control workers who worked at four separate facilities (a beer bottling plant, a water treatment plant, a meat packing facility, and an administrative facility) with no DEE exposure and are from the same local area in China as the exposed workers. These control workers were not occupationally exposed to particulates or other chemicals either known or suspected to be associated with genotoxicity, hematotoxicity, or immunotoxicity. Exposed workers were frequency-matched to controls by age (±5 years) and smoking status (never and ever). Demographic and lifestyle characteristics were obtained for each worker through an on-site questionnaire interview. Peripheral blood samples were collected from all workers immediately following their work shift as part of a health examination conducted by the local Center for Disease Control. Samples from the exposed and control groups were collected into 4 mL serum vacutainer tubes and processed by the same laboratory using the same procedures: within 4 h of collection and at room temperature, the serum tubes were centrifuged for 10 min at up to 1300 g and serum samples were taken off from the top of the clot before putting into a −80°C freezer for long-term storage. Informed consent was obtained from all subjects and the study was approved by Institutional Review Boards at the US National Cancer Institute and the National Institute of Occupational Health and Poison Control, China CDC.

An extensive exposure assessment survey was conducted in the diesel manufacturing facility where the DEE-exposed workers were employed, which included an assessment of several DEE constituents including elemental carbon (EC, the main exposure proxy for DEE), organic carbon (OC), and fine particulate matter ($PM_{2.5}$). Repeated personal air samples of EC, OC, and $PM_{2.5}$ were collected for a full work-shift using a cyclone, with an aerodynamic cut-off of 2.5 μm at a flow rate of 3.5 L/min, attached to the lapel near the breathing zone using quartz or Teflon filters. $PM_{2.5}$ was assessed by preweighing and postweighing the Teflon filters in an environmentally controlled weighing room using a microbalance at 1 μg accuracy. EC and OC were measured on the quartz filters using NIOSH Method 5040 (The National Institute for Occupational Safety and Health (NIOSH) (2003)). Exposure concentrations of EC ($\mu g/m^3$), OC ($\mu g/m^3$), and $PM_{2.5}$ (mg/m^3) were calculated using weights divided by the volume of air drawn through the filters. Exposure assessments were also conducted in a subset of five controls from unexposed factories, with the

exception of the beer bottling plant where no measurements could be obtained.

Among DEE-exposed workers, there were high correlations between EC and OC levels ($r_{sp} = .81$, $p < .0001$) but no correlation was observed between EC and $PM_{2.5}$ ($r_{sp} = -.14$, $p = .36$).

2.2 | Assays for micro-RNAs

MiRNAs were measured using the Fireplex circulating miRNA assay (Abcam, Inc., Cambridge, MA) that profiles miRNAs directly from serum without the need for a separate RNA isolation and purification step (Abcam, 2018). This approach is designed for high-throughput applications and reduces preanalytical variability. Results obtained from serum using this assay have been previously demonstrated to be highly correlated (>90%) with results from purified RNA and from qRT-PCR and sequencing-based approaches (Abcam, 2017, 2018).

We conducted a two-stage study. First, we conducted a small pilot study of six workers exposed to DEE and six controls in which 405 miRNAs were measured in serum using untargeted discovery panels to identify miRNAs that were expressed in our study population. These discovery panels included miRNAs with known expression in serum and reflected diverse biologic pathways. The initial differences of the measured miRNAs in serum levels between the exposed and control workers in the pilot study were assessed based on non-parametric tests (Wilcoxon test) and expression fold changes to select potential informative miRNAs evaluated in the second stage or primary study.

A standard laboratory protocol from the manufacturer was followed for the miRNA circulating assay (Abcam, 2018). Briefly, 40 μL serum was added to digestion buffer and incubated to lyse cells. Subsequently, miRNAs were hybridized into hydrogel particles. After labeling and amplification of target miRNAs, rehybridization to the original capture particles was performed on the fluorescent target, which was then scanned using a standard flow cytometer. Hemolysis markers (miR-451a and miR-486-5p) were assessed, and all serum samples had negative results suggesting no red blood cell contamination (Pritchard et al., 2012). Signals (expressed in arbitrary units, A.U.) were performed geNorm-based normalization by identifying the evaluated probes with the most stable expression levels across samples (miR-19b-3p, miR-92a-3p, and miR-17-5p in our study) and using the geometric mean expression of these miRNAs to normalize each sample for each miRNA target (Mestdagh et al., 2009).

We further used MetaCore (GeneGo) to conduct gene enrichment analysis by pathway maps with in silico predicted targets from TargetScan (v7.1; Agarwal et al., 2015; Cirillo et al., 2017). Predicted mRNA targets with a total context score of <−0.2 were selected for pathway enrichment analysis. Noteworthy pathways were selected based on an FDR <0.05.

Assay quality control (QC) was conducted by including 12 blind duplicate samples from six different QC subjects in the assay batches. Coefficients of variation (CV) of each evaluated miRNAs were calculated based on the blind duplicate samples. Of the 44 evaluated miRNAs, 38 were found to have CV < 40% and were included in the statistical analyses (Table S2).

2.3 | Statistical analyses

Differences in demographic and lifestyle characteristics between DEE-exposed and control workers were evaluated using Wilcoxon tests for continuous variables and Chi-square tests for categorical variables. Normal probability plots indicated that miRNA expression had log-normal distributions. Therefore, linear regression models using the natural logarithm (ln) of each miRNA were used to test for differences between exposed workers and controls. To evaluate exposure–response relationships with miRNA, EC, OC, and $PM_{2.5}$ were categorized using a four-level ordinal variable (controls and exposure tertiles among exposed workers). All models were adjusted for age (continuous) and ever smoking status (never/ever). Additional covariates included in the final models were current alcohol use (yes/no), recent respiratory infection in the previous month (e.g., flu), and body mass index (BMI; kg/m^2) if they were significant at $p < .05$ or there was evidence of confounding (i.e., a change in the β-coefficient > 10%). We used the false discovery rate (FDR) to account for multiple testing and considered a finding (p values of DEE exposed vs. controls or p_{trend}) with an FDR value <0.20 as noteworthy (Benjamini & Hochberg, 1995). FDR values were computed from adjusted p values using the Benjamini–Hochberg method (Haynes, 2013). All analyses were conducted in the SAS/STAT software, version 9.4.

3 | RESULTS

In the pilot stage, 405 miRNAs were measured in serum using untargeted discovery panels on samples from six diesel engine workers and six controls (Table S1). A total of 44 miRNAs from this initial screening were selected for further evaluation in the primary study of 45 workers exposed to DEE and 46 controls.

The demographic characteristic of the 45 DEE-exposed and 46 control workers are presented in Table 1 and show comparable distributions for age, BMI, smoking status, current alcohol use, and recent infection. Smoking intensity, smoking duration, and pack-years among ever smokers were also shown no statistical difference between DEE-exposed workers and unexposed controls. Workers exposed to DEE had a mean employment duration of 19.5 years. Unadjusted and background-adjusted DEE constituents (i.e., EC, OC, and $PM_{2.5}$) concentrations are presented in Table 1. There was a wide range of exposure to EC (Median, range: 47.7, 6.1–79.7 $\mu g/m^3$). Background-adjusted mean levels of EC, OC, and $PM_{2.5}$ in the exposed workers were 44.0 ± 18.4 $\mu g/m^3$, 64.0 ± 19.0 $\mu g/m^3$, and 0.1 ± 0.07 mg/m^3, respectively (Table 1).

Of the 38 miRNAs that were evaluated (Table S2), serum levels of four miRNAs [miR-191-5p ($p = .003$), miR-93-5p ($p = .03$), miR-423-3p ($p = .02$), and miR-122-5p ($p = .01$)] were significantly lower in DEE-exposed workers compared to unexposed controls.

TABLE 1 Demographic characteristics of diesel engine exhaust exposed and control workers.

	Controls ($n = 46$[a])	Exposed ($n = 45$[a])	p
Age, years, mean (SD)	42.7 (7.5)	41.9 (7.0)	.57[b]
BMI, kg/m^2, mean (SD)	24.8 (3.2)	24.8 (3.6)	.97[b]
Smoking status			
Ever, *n* (%)	39 (84.8)	38 (84.4)	.96[c]
Never, *n* (%)	7 (15.2)	7 (15.6)	
Smoking intensity among ever smokers, average cigs/day, mean (SD)	9.8 (8.3)	9.5 (8.5)	.77[b]
Smoking pack-years, mean (SD)	12.5 (12.5)	13.4 (10.2)	.45[b]
Current alcohol use			
Yes, *n*(%)	39 (84.8)	34 (75.6)	.27[c]
No, *n*(%)	7 (15.2)	11 (24.4)	
Recent infection (flu or respiratory infections in the previous month)			
Yes, *n* (%)	23 (50.0)	23 (51.1)	.92[c]
No, *n* (%)	23 (50.0)	22 (48.9)	
Work years in diesel factory			
Mean (SD)	NA	19.5 (7.5)	
Elemental carbon, μg/m^3			
Unadjusted, mean (SD)	11.1 (1.3)	55.1 (18.4)	
Background adjusted, mean (SD)	0 (NA)	44.0 (18.4)	
Organic carbon, μg/m^3			
Unadjusted, mean (SD)	68.7 (4.1)	132.7 (19.0)	
Background adjusted, mean (SD)	0 (NA)	64.0 (19.0)	
PM2.5, mg/m^3			
Unadjusted, mean (SD)	0.2 (0.07)	0.4 (0.07)	
Background adjusted, mean (SD)	0 (NA)	0.1 (0.07)	

Abbreviation: BMI, body mass index.
[a]Twelve subjects in pilot study and six subjects without enough serum samples available were excluded.
[b]*p*-value of Wilcoxon test.
[c]*p*-value of χ^2 test.

An additional miRNA (miR-92a-3p) was significantly higher in DEE-exposed workers compared to unexposed controls ($p = .049$). However, only miR-191-5p remained noteworthy after accounting for multiple comparisons (FDR <0.20; Table 2).

Two miRNAs [i.e., miR-191-5p ($p_{trend} = .001$) and miR-93-5p ($p_{trend} = .009$)] had a significant exposure–response relationship with the level of EC exposure after adjusting for multiple comparisons (FDR < 0.20; Table 2). The patterns of the exposure–response relationships for miR-191-5p and miR-93-5p in relation to the EC exposure levels were similar. Specifically, serum levels of both miRNAs were significantly lower among the highest exposed workers (i.e., exposed workers with EC levels in the third tertile) compared to control workers (−35%, $p = .003$ and −16%, $p = .005$ for miR-191-5p and miR-93-5p, respectively; Table 2). Only weak to moderate correlation was observed between levels of miR-191-5p and miR-93-5p ($r_{sp} = .36$ among workers exposed to DEE; Table S3; Akoglu, 2018). The association between EC and miR-191-5p remained statistically significant after additional adjustment for these miRNAs in the regression models (data not shown).

Of the five miRNAs that showed an association in DEE-exposed workers compared to control workers, the exposure–response for decreasing levels of miR-191-5p was consistent across increasing levels of EC, OC ($p_{trend} = .0002$, Table S4), and $PM_{2.5}$ ($p_{trend} = .004$, Table S5) after accounting for the FDR. In addition, levels of OC showed an exposure–response relationship with increasing levels of miR-93-5p ($p_{trend} = .006$, FDR = 0.12; Table S4). $PM_{2.5}$ had a significant exposure–response relationship with four of the five miRNAs (i.e., miR-191-5p, miR-93-5p, miR-122-5p and miR-92a-3p) after accounting for FDR < 0.20 (Table S5).

4 | DISCUSSION

We found significant differences in serum levels of five miRNAs between workers occupationally exposed to DEE and unexposed controls. Two miRNAs (i.e., miR-191-5p and miR-93-5p) also showed a significant exposure–response relationship with personal levels of EC and remained noteworthy after accounting for multiple comparisons.

TABLE 2 Exposure–response relationships between diesel engine exhaust exposure and miRNAs showed significant differences between exposed workers and controls.

	Controls ($n = 46$)		DEE exposed ($n = 45$)					First tertile (EC: 6.1–39.0 μg/m^3), ($n = 17$)				Second tertile (EC: 39.1–54.5 μg/m^3), ($n = 16$)				Third tertile (EC: 54.6–107.7 μg/m^3), ($n = 12$)					
miRNA	Mean[b]	SD[b]	Mean[b]	SD[b]	%[c]	p	FDR[a]	Mean[b]	SD[b]	%[c]	p	Mean[b]	SD[b]	%[c]	p	Mean[b]	SD[b]	%[c]	p	p_{trend}	FDR[a]
miR-191-5p[d]	400.7	174.5	309.9	164.5	−23	**.003**	**0.10**	333.0	207.5	−17	.1	322.6	135.8	−20	.07	260.0	128.9	−35	**.003**	**.001**	**0.04**
miR-93-5p[d]	747.1	128.2	692.6	149.6	−7	**.03**	0.29	713.7	126.2	−5	.3	720.5	192.2	−4	.5	625.4	96.0	−16	**.005**	**.009**	**0.18**
miR-423-3p[d]	13.3	7.1	10.0	5.9	−25	**.02**	0.25	10.0	6.2	−25	.1	10.3	6.8	−23	.1	9.7	4.7	−27	.2	.08	0.52
miR-122-5p[d]	1487.0	1444.6	854.5	685.1	−43	**.01**	0.24	637.4	416.1	−57	**.01**	1004.1	834.7	−33	.2	962.8	746.6	−35	.2	.1	0.52
miR-92a-3p[d]	1236.1	148.4	1311.6	204.8	6	**.049**	0.32	1341.5	238.4	9	**.03**	1311.0	196.0	6	.2	1270.3	171.9	3	.8	.3	0.66

[a]False discovery rate (FDR) values computed from adjusted p values using Benjamini–Hochberg method.
[b]Signals in arbitrary unit (A.U.).
[c]Percent difference in mean miRNA levels comparing DEE-exposed workers to controls.
[d]Adjusted for age, ever smoking, current alcohol, recent infection, and BMI.

MiR-191 plays an important role in cellular processes such as cell proliferation, differentiation, apoptosis, and migration (Mellios et al., 2008; Na et al., 2009; Nagpal et al., 2013; Zhang et al., 2011) by targeting important transcription factors, chromatin remodelers, and cell cycle associated genes. MiR-191 is a key regulator of naive, memory, and regulatory T cell homeostasis, following cytokine signaling that relies on STAT5 tyrosine phosphorylation; it also targets insulin receptor substrate 1 (*Irs1*), and increases IRS1 levels that induce cell death following stimulation (Lykken & Li, 2016). Several studies of lung cancer cell lines suggest miR-191 may play role in the development of lung cancer through the Wnt/β-catenin pathway (Mazieres et al., 2005; Xu et al., 2015; Zhou et al., 2020). Gene enrichment by pathway maps of miR-191-5p with their in silico predicted target mRNAs revealed several biological pathways implicated in cancers, including nonsmall cell lung cancers (FDR < 0.05) (Moody et al., 2017; Rybczynska et al., 1986; Theelen et al., 2016; Zhang et al., 2012; Zou et al., 2018). Notable pathways include Wnt/β-catenin signaling, NOTCH signaling, ERBB family signaling, FGFR3 signaling, IL-3 signaling via ERK and PI3K, and Endothelin-1/EDNRA transactivation of EGFR pathways.

MiR-93-5p is involved in posttranscriptional regulation of IL-8 and VEGF gene expression in a variety of cellular systems (Fabbri et al., 2016). A recent study showed that miR-93 may be involved in oxidative stress-induced mitophagy by synergistically inhibiting the translation of mitophagy receptors optineurin, nuclear dot protein 52, and phosphorylated mitofusin-2 (Zhang et al., 2021). Previous studies have also shown that miR-93-5p is involved in nonsmall cell lung cancer cell proliferation (Yang et al., 2018). On the other hand, pathway enrichment analysis showed that predicted mRNA targets of miR-93-5p were enriched in Ephrin-A signal transduction pathways, which has demonstrated or hypothesized contributions to modulatory processes controlling carcinogenesis and tumor progression (Pasquale, 2010; Xiao et al., 2020).

Recent studies found that environmental or occupational exposure to chemicals may be associated with alteration in miRNA profiles (Krauskopf et al., 2017; Mohr & Mott, 2015; Saliminejad et al., 2019; Vrijens et al., 2015). Exposure to DEE or diesel exhaust particles (DEP) is widespread, particularly in urban areas where traffic-generated particulate matter has become the principal source of air pollutants (Manchester-Neesvig et al., 2003). In 2012, the IARC re-classified DEE from Group 2A (probably carcinogenic to humans) to Group 1 (carcinogenic to humans) based on clear evidence of DEE being strongly associated with lung cancer in humans (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2014).

MiRNA can also leak into the circulation from tissue cytotoxicity, which could potentially explain that DEE is associated with alteration in different miRNAs. The updated miRNA Tissue Atlas (Keller et al., 2022) show that levels of the four miRNAs identified in our study (i.e., miR-191-5p, miR-93-5p, miR-423-3p, and miR-92a-3p) are relatively high in lung tissue, which is directly exposed to genotoxic and cytotoxic components of DEE. This finding could support that DEE is associated with lung cancer risk by promoting cytotoxicity in lung tissue, leading to alterations to circulating miRNA levels.

Although we do not have direct evidence from our study on whether DEE separately affects the stability of circulating miRNA themselves through genotoxicity, the components of DEE include well-characterized genotoxic agents such as PAHs and their nitrated species (nitro-PAHs; IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2014; Moller et al., 1993).

Although numerous studies have investigated the effect of DEE particles on changes in miRNA expression, the findings have been inconsistent (Cheng et al., 2020; Jardim et al., 2009; Krauskopf et al., 2018, 2019; Rodosthenous et al., 2016; Yamamoto et al., 2013). The reason may be that most of the studies used particles as DEE exposure marker (DEP) and few of them had conducted a detailed exposure assessment on EC, OC, and $PM_{2.5}$.

To the best of our knowledge, this study is the first to provide evidence that miR-191-5p responds to levels of DEE exposure in an exposure–response manner. A strength of this study was the detailed exposure assessment conducted among the diesel-exposed workers and their relatively high and wide range of air exposure levels of EC, OC, and $PM_{2.5}$ compared to control workers. The exposure assessment was further enhanced by the availability of personal monitoring data for these DEE constituents that enabled a detailed exposure–response analyses. In addition, all of the serum samples from exposed and control workers in our study were collected at the same time and were processed and analyzed using the same laboratories and protocols. Thus, any degree of misclassification of miRNAs in our study is expected to be nondifferential with respect to DEE exposure and would not be a likely explanation for the differences in serum levels that we observed.

Our study had several limitations. Because our study had a small sample size, the biological relevance of this finding will need further evaluation in larger studies (Drizik et al., 2020). While we controlled for common lifestyle characteristics (e.g., smoking, alcohol consumption) and demographic characteristics, it is possible that additional confounders may influence miRNA levels and be associated with DEE exposure. For example, we previously reported that total lymphocyte count and three lymphocyte subsets, and B cells were higher in DEE exposed compared to control workers (Lan et al., 2015). However, there was no correlation between miR-191-5p levels and lymphocyte subsets cell counts overall (r_{sp} ranging from −0.13 to 0.07) or in exposed workers (r_{sp} ranging from −0.01 to 0.08), suggesting that this finding is independent of effects of blood cell counts reported previously.

5 | CONCLUSIONS

In summary, our study showed that occupational exposure to DEE may alter circulating miRNAs including miR-191-5p, miR-93-5p, miR-423-3p, miR-122-5p, and miR-92a-3p in healthy workers. Our results suggest that miR-191-5p may be a plausible biomarker for an early biologic effect resulting from DEE exposure and could provide mechanistic context to the DEE and lung cancer association if replicated in future studies.

AUTHOR CONTRIBUTIONS

Wei Hu and Jason Y. Y. Wong conducted the data analyses and wrote the main manuscript text. Wei Hu, Yufei Dai, Dianzhi Ren, Huawei Duan, Yong Niu, Jun Xu, Wei Fu, Kees Meliefste, Baosen Zhou, Jufang Yang, Meng Ye, Xiaowei Jia, Tao Meng, and Ping Bin conducted the field study. Roel C. Vermeulen, Debra T. Silverman, Nathaniel Rothman, Yuxin Zheng, and Qing Lan designed the study. All authors reviewed the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data underlying this article are available in the article and its online supplementary material.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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