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Original Research

Glyphosate Use Predicts Healthcare Utilization for ADHD in the Healthcare Cost and Utilization Project net (HCUPnet): A Two-Way Fixed-Effects Analysis

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Abstract

There has been considerable international study on the etiology of rising mental disorders, such as ADHD, in human populations. As glyphosate is the most commonly used herbicide in the world, we sought to test the hypothesis that glyphosate use in agriculture may be a contributing environmental factor to the increase in healthcare utilization among individuals with diagnosed ADHD.

State estimates for glyphosate use and nitrogen fertilizer use were obtained from the USGS. We queried the Healthcare Cost and Utilization Project net (HCUPNET) for state-level hospitalization discharge diagnosis data on all patients for all-listed ADHD cases from 2007 to 2010. The least squares dummy variable (LSDV) method and within the method using two-way fixed effects was used to elucidate the relationship between glyphosate use and all-listed ADHD hospital discharge diagnoses.

A 1-kg increase in glyphosate use in one year positively predicts state-level all-listed ADHD discharge diagnoses the following year (coefficient = 5.54E-08, p<.01). A study of the effects of urbanization on the relationship between glyphosate use and ADHD indicates that the relationship is marginally significantly positive in urban U.S. counties (p<.025). Furthermore, total glyphosate use is strongly associated with total farm use of nitrogen fertilizers from 1992 to 2006 (p<.001).

Glyphosate use is a significant predictor of state healthcare utilization for ADHD, with the effect concentrated in urban U.S. counties. We draw upon the econometric results to propose unique and exploratory mechanisms, borrowing principles from soil and atmospheric sciences, for how glyphosate-based herbicides may be contributing to the rise of ADHD in all populations.

Keywords: glyphosate; nitrogen fertilizers; maize; nitrous oxide; ADHD; fixed effects; air pollution

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Introduction

Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder whose incidence worldwide has increased substantially in recent decades. The Centers for Disease Control (CDC) parent report data on ADHD among U.S. children indicates a sharp rise beginning in 2007 [1]. According to the CDC, symptoms of ADHD include "a persistent pattern of inattention and/or hyperactivity-impulsivity that interferes with functioning or development" [2]. Empirical evidence suggests that symptoms of ADHD are often associated with autism [3], further complicating and expanding the disorder profile. Yerys et al. [4] reported that ADHD symptoms in children with autism spectrum disorders (ASD) resulted in a greater autistic trait with more significant impairments in working memory and adaptive behavior. It was shown that deficits in executive function were more severe and persistent in patients with ADHD than with ASD [5]. Similarly, Nydén et al. [6] found adults with ADHD, in comparison to ASD and ADHD/ASD groups, experienced more significant neuropsychological impairments in exercises designed to measure intellectual ability along with attention and executive function.

Much focus has been devoted to identifying etiological factors underlying the disorder. Emerging genetic links to ADHD are promising [7, 8] but require replication in diverse populations. Xu et al. [9] have suggested that ADHD is associated with epigenetic aberrations among dopamine receptor and histone-modifying genes, suggesting the possible influence of external causes like secondhand smoke [10, 11] on disorder etiology. However, current cigarette smoking among U.S. adults has been declining in both genders between 2005 and 2013 [12], suggesting the existence of other external influences. We hypothesized that there may be a link between the rise in ADHD and the parallel rise in glyphosate exposure from agricultural use, whether through air, water, or food sources.

Glyphosate (N-phosphonomethylglycine) has become the most commonly used herbicide in U.S. industrial agriculture [13]. Its use has grown significantly with the development of crops genetically engineered to tolerate the herbicide [14] in part because of the appearance of glyphosate-resistant weeds. "Triple-stacked" corn is a hybrid corn variety that expresses three transgenic events simultaneously in the same plant, including the following: 1) the CP4 EPSPS (5-enolpyruvulshikimate-3-phosphate synthase) protein that adds resistance to the herbicide glyphosate, 2) Cry1Ab protein to protect against European corn borer (Ostrinia nubilalis), and 3) Cry3Bb1 protein to protect against corn rootworm (Diabrotica spp.) [15]. Given the functional intimacy of these two variables, we surmise that genetically modified corn and glyphosate could be external factors contributing to the increase in ADHD worldwide.

In their work, Ohno et al. [16] found that certain *Bacillus thuringiensis* (BT) proteins, specifically the Cry1Aa and Cry1Ab, when digested and thus fragmented in simulated gastric fluid, induced histamine release from rat mast cells. With Cry1Aa this effect was noted to be most pronounced in a low-pH environment. Enhanced enteric histamine release could begin the neural-enteric crosstalk cascade that could eventually adversely impact dopaminergic activity [17], and dopamine has been implicated in attention-related neural networking [18]. In addition to Bt protein, glyphosate itself could also be a contributing factor to the increasing prevalence of ADHD.

Glyphosate has been shown to disrupt cytochrome P450 (CYP) enzymes [19], which, among many other effects, can inhibit activation of vitamin D3, which depends on CYP enzymes in both the liver and kidneys [20]. Vitamin D regulates serotonin synthesis in the brain [21], and reduced central nervous system serotonergic activity has been implicated as a risk factor in ADHD [22].

Our preliminary investigations led us to consider the complicit role of nitrogen cycling as an effect modifier explaining the potential relationship between glyphosate use and ADHD. We became aware that glyphosate use on crops such as maize necessitates an increased application rate of nitrogen fertilizers, because glyphosate disrupts the uptake of nitrogen by plants [23]. We also propose that glyphosate disrupts the soil bacteria, leading to changes in the way nitrogen is handled in the soil. The action of microbes in the soil and water can cause the release of nitrous oxide (N_2O) into the air, which may lead to toxic effects on human physiology. The dopaminergic system was shown to mediate the antinociceptive effects of N_2O [24], and levels of central neurotransmitters, dopamine (DA), and norepinephrine (NE) were significantly elevated after repeated N_2O exposure in CD-1 mice [25]. Dysregulation of both catecholamine systems has been implicated in ADHD [26].

Our analysis has focused on state-level data for ADHD hospital discharge diagnoses and corresponding information about glyphosate use, nitrogen fertilizer use, and industrial and food use of maize, plus associated N_2O emissions. We use herbicide-resistant weed event statistics for further clarification on glyphosate use. Our objectives are shown as an empirical schematic in Fig. 1.

We have uncovered a pattern that is challenging to explain: glyphosate use significantly positively predicts glyphosate resistance events in weeds prior to 2007, but not subsequently. We suspect that a modification in the formulation and the use of maize as a residual cover crop in agriculture caused a widespread suppression of weed resistance development, while at the same time preceding

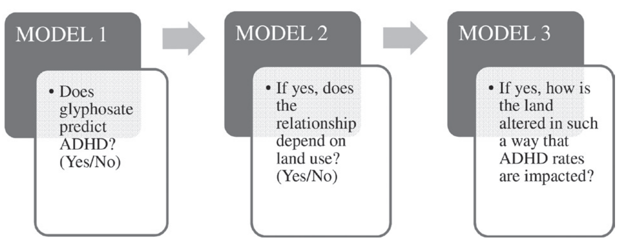

Fig. 1. The empirical schematic for studying the association between glyphosate use and ADHD.

an increase in ADHD in the population. We expect that the two are related, and we propose hypotheses to explain this relationship.

Methodology

Herbicide Use

Pesticide use was determined using data from the United States Geological Survey [27]. The USGS uses the estimated pesticide use rate (known as EPest rate) to determine pesticide usage rates for 39 pesticides among 76 crops and in 304 crop-reporting districts (CRD) in the United States from 1992-2009. Pesticide usage was defined as pounds applied per harvested-crop acre in the CRD. The proprietary pesticide use survey data for each CRD was obtained from GfK Kynetec, Inc. These data were normalized to the harvested acreage in nearby counties to arrive at county estimates of pesticide use rate. Harvested acreage is non-specific and includes the crops themselves, the soil on which the crops were grown, and the air above the acreage. In our analysis we have summarized EPest high county estimates (we also use low county estimates for purposes of replication) to generate state-level pesticide use for each year in 2006-09 – a practice recommended by the USGS [28]. The difference between high and low county estimates is that high county estimates include more counties. We have also included use of other herbicides – arbitrarily selecting atrazine, halosulfuron, and clethodim – as referent herbicides from the most prevalent groups of herbicide resistance, including Photosystem II inhibitors, acetolactate synthase (Als) inhibitors, and acetyl-Coenzyme A carboxylase (ACCase) inhibitors, respectively.

Nitrogen Inputs

We used county-level estimates of nitrogen fertilizer use to compile state statistics for farm use of nitrogen fertilizer between the years 1992 and 2006. The selection protocol can be found in [29].

State-level estimates of total food and industrial use of maize were gathered from data published by the University of Nebraska-Lincoln [30]. The data was derived from the “Feed Grain Database: Yearbook Tables, Corn: Food, Seed, and Industrial Use,” Table 31 from the Economic Research Service at the USDA [31]. State data were expressed as a percentage of total food and industrial use of maize grain in the U.S.

Nitrous Oxide (N_2O) Emissions

Agricultural N_2O emissions were obtained from the U.S. Energy Information Administration [32]. Emissions are expressed as million metric tons carbon dioxide equivalent. The EIA classifies emissions according to the following sources: agriculture, energy, industry, and waste management. To account for all sources, we expressed agricultural emissions as a percentage of total N_2O emissions.

Herbicide Resistance Events

Statewide data for herbicide-resistant weeds was obtained from the International Survey of Herbicide Resistant Weeds (ISHRW) in August 2014 and replicated in April 2015 [33]. We used four herbicide-resistant categories, according to the sites of action resisted. To measure the effect of weeds resistant to EPSP synthase inhibitors (which account for 9.6%, or 14 out of 146, of total herbicide-tolerant weeds in the United States at the time of this publication), we chose to include in our model the herbicides that accounted for at least 10 percent of the total cumulative resistant weeds in the United States at the time of this publication as reported by ISHRW. Therefore, we included the following herbicide groups, showing the percent of the total resistant weeds in the United States in parentheses: ALS inhibitors (31.5%, or 46 out of 146), Photosystem II inhibitors (17.8%, or 26 out of 146), and ACCase inhibitors (10.3%, or 15 out of 146). However, given that one weed can possess multiple sites of resisted action, we used the number of resisted sites of action for each of these groups in each state. To account for groups with less than 10 percent of the total cumulative resistant weeds, we expressed data for herbicide weed resistance in each group of interest as a percentage of the total cumulative weed resistance events in each state for the period 2000-12. Data before 2000 was incorporated into the cumulative analysis.

Hospitalization Data

We queried the Healthcare Cost and Utilization Project (HCUPNET) State Inpatient Database to identify state-level hospital discharge data trends for all-listed diagnoses of mental disorders [34]. The number of states reporting such data to HCUPNET was 37, although not all states reporting provided data for every year. The time period of interest was 2007 to 2010. Records for attention-deficit, conduct, and disruptive behavior disorders in HCUPNET began in 2007. For the all-listed diagnostic category we searched for the number of discharges that received a diagnosis of attention-deficit, conduct, and disruptive behavior disorders (ADHD). HCUPNET defines all diagnoses as being “… the principal diagnosis plus additional conditions that coexist at the time of admission, or that develop during the stay, and which have an effect on the treatment or length of stay in the hospital.” All diagnoses that patients receive while admitted to the hospital are assigned to an International Classification of Disease, 9th Revision – Clinical Modification (ICD-9) code. The ICD-9 codes that are included in the clinical classification software (CCS) diagnosis of attention-deficit, conduct, and disruptive behavior disorders are shown in Table S1 (data not shown) along with a translation to respective codes in the Diagnostic and Statistical Manual for Mental Disorders IV, Text Revision.

Importantly, since children and adults with ADHD have significantly higher rates of healthcare utilization [35-37] (including hospitalizations), hospital discharge diagnoses may be an important discriminatory marker of ADHD in diverse populations, potentially screening out "pure" or less severe ADHD, which are said to be a minority of cases [37] and even "highly atypical" [38]. It was our goal to test whether herbicide use predicts healthcare utilization for ADHD (i.e., likely significant ADHD impairment). We were not seeking to establish or utilize state-based prevalence of ADHD, as community prevalence rates employ differing methodologies and would, therefore, be unreliable [39]. To control for the central nervous system comorbidities that are often associated with an ADHD diagnosis [35-37], we have normalized the number of all-listed ADHD hospital discharges to a percentage of total discharges for all-listed mental disorders recorded in HCUPNET.

Hospitalization discharge data for all-listed ADHD diagnoses were also categorized according to the location of the patients' residence, as a percentage of the total all-listed ADHD hospital discharges. HCUPNET provided four categories in this respect: large central metro, large fringe metro (suburbs), medium and small metro, micropolitan and noncore (rural). We have created four categories, called metropolitan, fringe metropolitan, urban, and rural, to mirror the glyphosate and nitrogen fertilizer usage urbanization coding system mentioned previously (Table 2). We also gathered data on age and gender of patients with an all-listed ADHD discharge.

Least Squares Dummy Variable (LSDV) Regression

Panel regressions were performed using the LSDV in *plm* package in R, version 3.1.3 [40]. Two-ways within estimation was also used to confirm the LSDV estimations and all model diagnostics and was used solely when time (T) for each subject was greater than four years. The LSDV method creates dummy variables for both state and time, while the two-ways within effect estimations used deviations from group means. Fixed effects data were checked for heteroskedasticity in R using the Breusch-Pagan (BP) test (package *lmtest*) [41]. Data were also tested for serial correlation using the Durbin-Watson (DW) statistic [40, 41]. We chose the Fisher-type test in Stata 11.1 [42] to check for unit roots in unbalanced panels for each model since the test uses Newey-West standard errors to account for any serial correlation present among the residuals in each model. To our knowledge, an equivalent test was not available in R.

The Augmented Dickey-Fuller (ADF) test (package *tseries*) [43] was used to test for unit roots in Model 3 panel sets. We used the Arellano variance covariance (*vcov)* HC function covariance estimator [44] to control for heteroskedasticity and serial correlation in all models. We used student's paired t-test when making comparisons between age and urban subsets of ADHD hospital discharge among available HCUPNET states during our study period (2007-10). Data were first tested for normality using Shapiro-Wilk test. Unless otherwise specified, we define significance at the .05 level for all statistical testing performed, while a .10 level was used for model diagnostic testing (i.e., BP, DW, ADF, Fisher test). Graphical output was performed using R and GraphPad Prism 6 Demo for Windows, GraphPad Software, La Jolla California USA (www.graphpad.com).

Model 1

Our first model tested our hypothesis that glyphosate use in one year predicts all-listed ADHD hospital discharge diagnoses the following year. In addition to glyphosate and maize use, we have identified four time-variant covariates, or regressors, including other societal and health care-related variables that have been associated with the disorder and could explain the increase in ADHD diagnoses. Furthermore, we have included glyphosate resistance to clarify the herbicide's effect on human health. We subjected each covariate, excluding our variables of interest, glyphosate use, and food/industrial maize use, to a single linear ordinary least squares model for each year from 2007 to 2010, inclusive. If a significant relationship was noted in any year at the .05 alpha level, we included the covariate in the final fixed effects model. Model 1 is:

$$Y_{it} = c_1 + \alpha_i + v_t + \sum_{m=3}^{M} \beta_{i,t-1}^{m} X_{i,t-1}^{m} + + \sum_{p=1}^{P} \beta_{i,t}^{p} X_{i,t}^{p} + \varepsilon_{it} \quad (1)$$

…where Y_{it} is the proportion of all mental disorders for which ADHD was a discharging condition in state *i* and year *t*, c_1 is a constant, α_i represents a state fixed effect to control for permanent differences between states, and v_t denotes a time fixed effect, $X^{m}_{i,t-1}$ is a (N_1 *x* 1) column vector representing an individual lagged covariate *m,* with *m* = 1,. . . , *M,* and N_1 is the total number of state-time observations, $X^{p}_{i,t}$ is a (N_1 *x* 1) column vector representing an individual contemporaneous covariate *p*, with *p* = 1, . . . , *P*, and ε_{it} captures unobserved heterogeneity in state *i* and year *t*. The parameters to be estimated and reported include $\beta^{m}_{i,t-1}$ and $\beta^{p}_{i,t}$. The states of Nevada and Hawaii were excluded in this model since there were no weed resistance or glyphosate use data, respectively, for the years of interest. All covariates in model 1 are explained in Table 1.

Model 2

Our second model – an *ad hoc* analysis – sought to test the hypothesis that if glyphosate is significantly contributing to ADHD, there would be regional influences underpinning this relationship. That is to say that glyphosate

Table 1. Identification, justification, and description of the covariates included in Model 1.

Model Notation	Covariate	Description	Citation
Contemporaneous			
p = 1	Health care access [45]	defined by the number of hospitals in each state, expressed as a percentage of total hospitals in the United States	[46]
p = 2	Economic [47]	defined by the annual home price index in each state, expressed as an average of the index over the four quarters of each year	[48]
p = 3	Population [49]	defined by the percent of the total United States population that resides in each state in each year	[50]
Lagged			
m = 1	Precipitation Lag [51]	defined as the deviation from the average annual precipitation in each state. For example, a reading of .88 would indicate that the precipitation in a given state during a given year was 88% of its average precipitation, with the average being defined by the base period, 1901-2000; (Parameter: Precipitation, Time Scale = 12 months, Month= December)	[52]
--	Herbicide resistance	See Text	[33]
m = 2, m = 3	Herbicide use/Maize Use	See Text	[27, 30]

is a main input in the agricultural industry, which largely has existed in rural (i.e., less populated) parts of the country, although inroads have been made in sustaining and expanding adaptive metropolitan agriculture systems [53]. To study this, we have categorized all counties from the USGS glyphosate use estimates into four codes from the USDA Economic Research Service Rural-Urban Continuum Codes [54] to generate four data points for each HCUPNET state in each year. The state of Hawaii was excluded since no estimates of glyphosate use were available. These data were then matched to the ADHD data in HCUPNET, according to the model specifications

Table 2. The coding system that was used in Models 2 and 3.

	USDA Economic Research Service Rural-Urban Continuum Codes (RUCC)[a]		HCUPNET ADHD (patient residence)
Model Set	*Code*	*Description*	
k = 1	*j* = 0	Central counties in metro areas of 1 million population or more (1993 only)	Large central metro
	j = 0		
	j = 1	(Fringe, only 1993) counties in metro areas of 1 million population or more	Large central metro
	j = 1		
k = 2	*j* = 2	Counties in metro areas of 250,000 to 1 million population	Large fringe metro
	j = 3	Counties in metro areas of fewer than 250,000 population	Large fringe metro
k = 3	*j* = 4	Urban population of 20,000 or more, adjacent to a metro area	Medium/Small metro
	j = 4		
	j = 5	Urban population of 20,000 or more, not adjacent to a metro area	Medium/Small metro
	j = 5		
	j = 6	Urban population of 2,500 to 19,999, adjacent to a metro area	Medium/Small metro
	j = 6		
	j = 7	Urban population of 2,500 to 19,999, not adjacent to a metro area	Medium/Small Metro
	j = 7		
k = 4	*j* = 8	Completely rural or less than 2,500 urban population, adjacent to a metro area	Micropolitan and noncore (rural)
	j = 8		
	j = 9	Completely rural or less than 2,500 urban population, not adjacent to a metro area	Micropolitan and noncore (rural)
	j = 9		

[a]Continuum codes changed from 1993 to 2003.

in Table 2. The other three predictors were unchanged from Model 1. Hospitalization data by location of patient residence and patient age was masked (*) in HCUPNET if less than or equal to 10 discharges or fewer than two hospitals were reported, and these data were removed from the analysis. Beginning in 2007, HCUPNET began revising data on location of patient residences to accommodate the specifications provided by the Economic Research Service at the USDA. Our four models were:

$$Y_{it}^{k} = c_2^k + \alpha_i^k + v_t^k + \gamma_{it}^k X_{it} + + \sum^{M=2} \gamma_{i,t-1}^{m,k} X_{i,t-1}^{m} + \beta_{i,t-1}^{k} G_{i,t-1}^{k} + \varepsilon_{it}^{k} \quad (2)$$

…where each model corresponds to one of four unique k values; Y_{it}^{k} is the proportion of total all-listed ADHD disorders in state i and year t that occurred among patients residing in a particular county classification code (k = 1, 2, 3, 4); c_2, α_1, and v_t are as defined in the previous sub-section for each model k; X_{it} is a contemporaneous continuous variable (i.e., population percent, hence the p superscript equals 1 and is therefore omitted for simplicity) in state i and year t as in model 1; $X_{i,t-1}^{m}$ denotes one of two *lagged* variables predicting ADHD discharges; and ε_{it}^{k} captures unobserved heterogeneity in state i and year t for model k. Our main predictor of interest, glyphosate use ($G_{i,t-1}^{k}$), is expressed in kilograms as the previous year's total use in counties categorized only by urbanization code k in state i. The 16 parameters to be estimated include γ_{it}^{k}, $\gamma_{i,t-1}^{l,k}$, $\gamma_{i,t-1}^{2,k}$, and $\beta_{i,t-1}^{k}$ for k = 1, 2, 3, and 4. The study significance was adjusted for multiple comparisons across model categories; the revised p-value of 0.0125 was used to determine significance.

Model 3

Third, we tested our hypothesis that glyphosate use could be indirectly altering the land biota to impact the rise of ADHD. We assessed the influence of county level estimates of glyphosate application on county level estimates of farm nitrogen fertilizer use between 1992 and 2006. The hypothesis is that increasing amounts of glyphosate use would perturb soil such that greater farm use of nitrogen fertilizer would be needed. We summed glyphosate and farm nitrogen use for all USDA county continuum codes between 1992 and 2006. We then organized our panel sets by study categories set forth in Table 2. We focused our attention to glyphosate use in urban counties given our results with the Model 2 set. For comparison, we also analyzed results for fringe metro counties. Our two models were:

$$Z_{j,t}^{k} = c_3^k + \alpha_j^k + v_t^k + \beta_{j,t}^{k} X_{j,t}^{k} + \epsilon_{j,t}^{k} \quad (3)$$

…where each model corresponds to one of two unique k values, $Z_{j,t}^{k}$ is a (N_3 x 1) column vector (n is the number of RUCC, and N_3 is represented by (n*t)) and denotes the farm use of nitrogen-based fertilizers (in kilograms) in the RUCC county code j with classification category k in year t, c_3^k is a constant that varies by model, α_j^k is a RUCC fixed effect, v_t^k denotes a time fixed effect, $X_{j,t}^{k}$ indicates glyphosate use estimate (in kilograms) in the RUCC county code j with category k in year t, and $\in_{j,t}^{k}$ captures unobserved heterogeneity in county code j in year t. The two parameters to be estimated and reported include $\beta_{j,t}^{k}$, where k = 2 and 3. These estimates generate a comparison of the effect of glyphosate use in fringe metro and urban counties upon the application of nitrogen-based fertilizers. The study significance was adjusted for two comparisons across model categories; the revised p-value of 0.025 was used to determine significance for the glyphosate estimates.

Results

Herbicide Use

Estimated national glyphosate use increased 36.2%, or 26,795,398 kg from 2006 to 2009, whereas use increased 59.6%, or 26,668,369.50 kg in all available HCUPNET states reporting data on ADHD hospital discharges for available years between 2007 and 2010. The average estimated glyphosate use over the HCUPNET reporting states is shown in Fig. 2a. Some HCUPNET states did not have data for all years of interest (2007-10), including Illinois and New Mexico (2009, 2010) and New Hampshire (2007-09).

Nitrogen Inputs

USGS county sums of farm use of nitrogen fertilizer show that the greatest relative percentage increase occurred in those counties with a USDA rural-urban continuum code of either 3 or 4 (64 and 49%, respectively). Total U.S. food and industrial use of maize grain increased by 2 billion bushels between 2006 and 2009.

Agricultural N_2O Emissions

According to the U.S. Environmental Protection Agency, nitrous oxide (N_2O) accounted for 5% of all U.S. greenhouse gas emissions. Man-made sources of N_2O include agriculture, energy, industry, and waste management practices, with agricultural soil management accounting for 75% of U.S. N_2O emissions. Because of its long half-life, N_2O is thought to be a source with a global warming potential of approximately 300 times that of other greenhouse gases, e.g., carbon dioxide [55].

Total N_2O agricultural emissions have been increasing since 1990. Total agricultural emissions have increased as a percentage of total N_2O emissions by 9.20%, or about 12.3 million metric tons from the period 1990-2009 [32].

Direct agricultural soil N_2O emissions increased 9.1% as a percentage of total N_2O emissions, or about 9 million

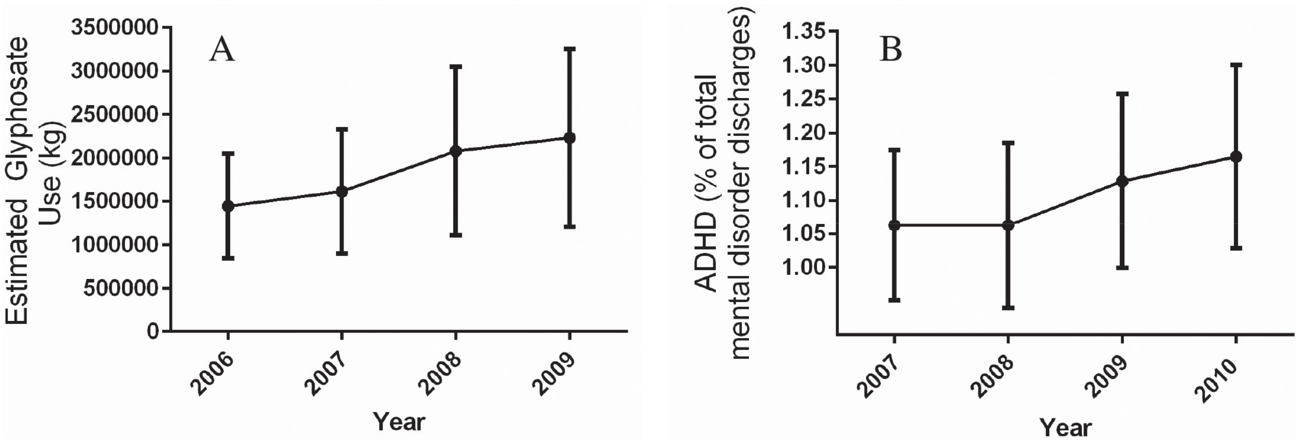

Fig. 2. Glyphosate use and all-listed ADHD hospital discharge diagnoses. (A) Average glyphosate use (in kilograms) in the HCUPNET states in Model 1 (Nevada and Hawaii excluded). Data are presented as average percentages with 95% confidence interval across all reporting states for each year indicated (2006-09). (B) Average all-listed hospital discharges for ADHD, as a percentage of total all-listed mental health discharges in HCUPNET reporting states (excluding Nevada and Hawaii) for 2007-10 used in Model 1. Data are presented as average percentages with 95% confidence interval across all reporting states for each year indicated.

metric tons carbon dioxide equivalent, from 1990-2009. Almost half of that normalized increase (48.77%) came during the period of the current study (2006-09). Most of that increase between 2006 and 2009 was attributable to above- and below-ground crop residues. Use of high residue crops in soil management is a growing trend in agriculture [56]. Indirect agricultural soil N_2O emissions increased 11.28% as a percentage of total N_2O emissions, or about 2 million metric tons carbon dioxide equivalent, between 1990 and 2009. Most of this increase is attributable to soil leaching and runoff.

Herbicide Resistance Events

Using two-way fixed effects we can validate that the use of herbicides with contact modalities of action does significantly predict class resistance between 2000 and 2010 (*data not shown*). However, during our study period from 2006 to 2009, glyphosate use does not significantly predict EPSP synthase inhibitor resistance events the following year in states (N = 188) (unadjusted estimate: 4.29e-09±7.04e-09, p-value: 0.54), or in HCUPNET states (adjusted estimate: 7.55e-09±1.24e-08, p-value: 0.54). Therefore, the development of herbicide resistance (i.e., herbicide application on vegetation) among contact herbicides like glyphosate is insignificant and unrelated to any link between use and disorders of interest between 2007 and 2010, and this data is confirmed by other reports [57].

Hospitalization Data

Fig. 2b shows the increase in ADHD discharges across HCUPNET reporting states (excluding Nevada and Hawaii, as these states had no weed statistics or glyphosate use data) from 2007-10. Data are expressed as ADHD discharges as a percent of all-listed hospitalization discharge for all mental disorders.

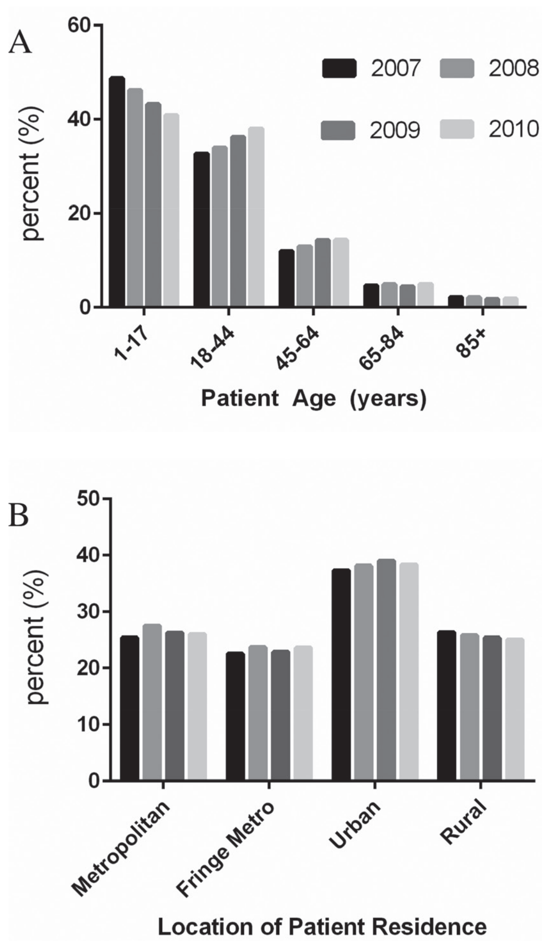

Fig. 3. Hospital discharges for ADHD (CCS diagnostic category 652) by (A) patient age and (B) location of patient residence. Data are presented as the average percentage for available HCUPNET reporting states for each year indicated and may therefore not add to 100%. Masked data (*) in HCUPNET – or data for which there were 10 or fewer patient discharges or fewer than two hospitals – were excluded from analysis. The state of Hawaii is excluded from the descriptive statistics reported for the ADHD population.

Table 3. Effect of herbicide use and other covariates on ADHD hospital discharges for the identified years.

Covariate		Linear Model (criteria variable for inclusion, p<.05) 2008				2010				Time and State Fixed Effects (2007-2010)		
		N	r^2	Estimate	Std Error	**N**	r^2	Estimate	Std Error	N_I [a]	**Estimate**	**Std Error**
Food/Industrial Use - Maize		--	--	--	--	--	--	--	--	127	2.70E-02	0.013 *
Pesticide												
Glyphosate	Amino Acid synthesis inhibitor	--	--	--	--	--	--	--	--	127	5.54E-08	1.68e-08 **
Atrazine	Photosystem II inhibitor	31	0.04	6.98E-08	6.28E-08	32	0.04	6.92E-08	5.93E-08	--	--	--
Clethodim	ACCase inhibitor	31	0.03	1.11E-05	1.11E-05	32	0.02	5.96E-06	7.35E-06	--	--	--
Halosulfuron	Acetolactate synthase inhibitor	31	0.03	-7.75E-05	7.82E-05	32	0.01	-3.18E-05	6.12E-05	--	--	--
Home Price Index		31	0.09	-4.41E-01	2.66E-01	32	0.02	-2.84E-01	3.97E-01	--	--	--
Population		31	0.17	-5.41E-02	0.022*	32	0.14	-5.53E-02	0.02499*	127	-4.11e-01	2.81e-01
Health Care Access		31	0.07	-5.03E-02	3.35E-02	32	0.05	-4.82E-02	3.81E-02	--	--	--
Precipitation Lag		31	0.14	6.71E-01	0.3109*	32	0.01	1.96E-01	3.96E-01	127	1.13e-02	4.50e-02

** p<0.01, * p<0.05. [a] N_I is the total number of state-time observations.

We found distinct trends emerging from a breakdown of all-listed ADHD discharges by patient age in HCUPNET reporting states from 2007 to 2010. Persons aged 1-17 years accounted for an average of 48.8% of the all-listed ADHD discharges in 2007, but that percentage decreased to 40.9% by 2010 – a statistically significant drop (p-value = 0.01, 95% CI: 1.67-14.01), while adults aged 18-44 gained as a percentage of total all-listed ADHD discharges, climbing from 32.7% to 38.0% across all HCUPNET reporting states (p-value < 0.05, 95% CI: -9.90- -0.74) (Fig. 3).

Hospital ADHD discharge diagnoses by gender revealed that the majority were males (*data not shown).* Urbanization data among both HCUPNET reporting states and national HCUPNET statistics indicate that the greatest percentage of all-listed ADHD diagnoses occur among persons living in medium and small metro (urban populations) areas (Fig. 3).

Fixed Effects

Model 1

The results from the simple ordinary least squares model screening process are presented in Table 3. Population was correlated with all-listed ADHD in both 2008 and 2010. Precipitation as a lagged indicator was correlated with all-listed ADHD in 2008. Therefore, population and precipitation were added to the two-way fixed effects final model. Specifically, P = 1 and M = 3 given these screening results. None of the arbitrarily selected herbicides passed the initial screening at either USGS estimate.

The Durbin-Watson test for panel data indicates marginal serial correlation (DW = 1.721, p-value = 0.067) among the residuals. The Breusch-Pagan test shows the presence of marked heteroskedasticity in this model (BP = 51.95, df = 4, p-value = 1.41e-10). The Fisher-type test indicated the presence of stationarity in at least one panel for all covariates.

For every kilogram increase in glyphosate use (high estimate) the prior year, there is a 5.54E-08% increase (p<0.01) in all-listed ADHD hospital discharges as a percent of all mental health disorder discharges in each HCUPNET reporting state the next year (2007-10), and this effect is seen in the absence of glyphosate use significantly predicting EPSP synthase inhibitor resistance events (Table 3). Fig. 4 shows the heteroskedastic trends in this model. These findings were replicated when *low* USGS herbicide estimates were used for this model as well as when only the hyperactivity subtype (ICD-9 code 314.01) was used, again normalized to a percentage of total mental health discharge diagnoses (*data not shown*).

For every percentage increase in maize for food and/or industrial use in the prior year, as a percentage of total U.S. maize for food and/or industrial use, there is a 0.027% increase (p<0.05) in all-listed ADHD hospital discharges as a percent of all mental health disorder discharges

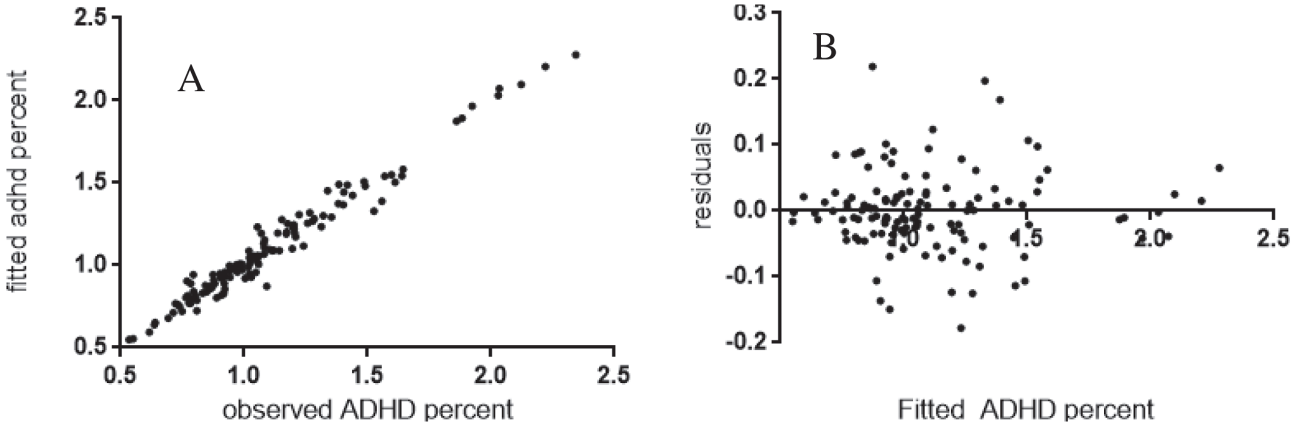

Fig. 4. Diagnostic plots for Model 1. Model diagnostics show marked heteroskedasticity within the center of distribution. (A) Observed all-listed ADHD percent discharges (N1 = 127) plotted against predicted percentages (B) Model residuals were plotted against fitted values. Fitted values were tabulated in R by subtracting residuals from observed values.

in each HCUPNET reporting state the following year (2007-10).

To further help inform our analysis on glyphosate as a single predictor for other mental disorders, we used a two-way fixed effects with the same HCUPNET states and years and found that glyphosate use does not significantly predict schizophrenia and other psychotic disorders during the earlier period in the decade when use predicts resistance (adjusted estimate: 2.00e-08±5.65e-08, p-value: 0.72), but *does* strongly predict them in the latter period when use does not predict resistance (2007-10) (unadjusted estimate: 9.18e-08±3.12e-08, p-value < 0.01).

Model 2

The urbanization data suggests that glyphosate's significantly positive relationship with all-listed ADHD hospital discharges is marginally significant in urban counties. As shown in Table 4, for every kilogram increase in glyphosate used in urban counties, the model predicts a 2.40e-06 percent increase in the percentage of all-listed ADHD hospital discharges that are attributable to patients living in these areas. The Durbin-Watson test indicated no serial correlation among the residuals in this sample (DW = 2.30, p-value = 0.96), and the data was determined to be heteroskedastic using the Breusch-Pagan test (BP = 9.90, df = 4, p-value = 0.042). The Fisher-type test indicated the presence of stationarity in at least one panel for all covariates.

We have performed additional iterations of this model to accommodate the flexible interpretations of the rural and urban continuum codes among government agencies (*data not shown*). The same results were noted. These results confirm the first model design and suggest that the model is robust to flexible continuum interpretations.

Model 3

The two-way within model fixed effects indicates that glyphosate use is associated with farm use of nitrogen fertilizers. In fringe metropolitan counties, the association is significantly positive (unadjusted estimate: 188.38±16.52, p-value < .001). Serial correlation was not present among the residuals (DW = 2.78, p-value = 0.99), and the data was homoskedastic (BP = 0.25, df = 1, p-value = 0.62). We used second-order differencing of both variables and confirmed no unit roots with the ADF test in this longer time series.

In the urban counties as we defined them, the association was significantly positive. (adjusted estimate: 150.53±24.91, p-value < .001). Serial correlation was not present (DW = 3.41, p-value = 1) and data were homoskedastic (BP = 0.21, df = 1, p-value = 0.64).

Table 4. Effect of urbanization on the relationship between glyphosate use and ADHD hospital discharges.

	Glyphosate use (2006-2009) and ADHD discharges (2007-2010)			
Category	N_2^k	r^2	Estimate	Standard Error
Metropolitan (*k* = 1)	88	0.01	-1.92e-06	4.00e-06
Fringe Metro (*k* = 2)	104	0.02	-6.94e-07	2.52e-06
Urban (*k* = 3)	123	0.08	2.40e-06	9.95e-07[§]
Rural (*k* = 4)	111	0.12	-4.17e-06	2.40e-06

§ corrected p<0.025

We differenced both variables twice to ensure stationarity in the urban time series.

Discussion

The current exploratory study suggests that glyphosate use may be contributing to all-listed ADHD hospital discharges in the United States. The main findings of the study include the following:

1. Lagged glyphosate use significantly positively predicts healthcare utilization among patients with an all-listed ADHD hospital discharge diagnosis in the 33 HCUPNET reporting states from 2007-10.
2. Glyphosate use strongly positively predicts glyphosate weed resistance events in states from 2001 to 2010, but not in the 33 HCUPNET reporting states or all available states (N = 47) from 2007 to 2010.
3. Our urbanization coding system revealed that glyphosate's positive association with ADHD remained marginally significant only in urban counties between 2007 and 2010, suggesting that urbanization may be a factor in the relationship.
4. Glyphosate use is significantly positively associated with farm use of nitrogen fertilizers between 1992 and 2006 in urban and fringe metro counties, suggesting that farm use of nitrogen fertilizers may be contributing significantly to the association between glyphosate use and all-listed ADHD hospital discharge diagnoses prior to 2007. There may be other land interactions that could be contributing to an increase in healthcare utilization among patients with an all-listed ADHD hospital discharge diagnosis after 2006.

The Link between Glyphosate, Nitrogen Inputs, and Soil Microbiota

Glyphosate's role as a mineral chelator in soil [58] could cumulatively dampen manganese bioavailability in soil. Manganese is important for nitrogen assimilation in plants. If less manganese is available, less nitrogen assimilation occurs. This would explain the strong positive association we have found between glyphosate and nitrogen-based fertilizers. This strong association could contribute to indirect agricultural N_2O emissions from leached anthropogenic nitrogen into urban drainage networks [59, 60].

The present findings suggest some kind of modification in the use of glyphosate around 2006. The ability for estimated glyphosate use alone to predict mental health disorders changed significantly from 2001 to 2010. Moreover, the inability of estimated glyphosate use to predict EPSP synthase inhibitor resistance events in the latter part of the decade, whereas it had in the earlier part of the decade, is evidence of a formulaic inconsistency [61].

Egamberdiyeva et al. [62] have shown that adding nitrification inhibitors such as oxalates inhibits net nitrification in calcareous soils. Furthermore, Wan et al. [63] demonstrated that ammonia-oxidizing archaea (AOA) were more responsive to a simazine herbicide application to agricultural soils. Denitrifying bacterial populations play a critical role in N_2O emissions from amended soil [64]. Therefore, increasing use of herbicides and adding nitrification inhibitors like oxalates to fertilization programs in the United States would theoretically result in an attenuation of the nitrification process that is the conventionally regarded rate-limiting reaction contributing to N_2O release from agricultural soils [65].

However, this inhibition of nitrification could potentially lead to nitrogen mismanagement in the soil, including an accumulation and potential release of soil ammonia (NH_3) after urea-based fertilizer application [66]. Compared to ammonia-oxidizing (AO) bacteria (AOB), AOA strains have a much higher substrate affinity for ammonia and oxygen and can therefore thrive in the crust of low-nutrient microaerophilic soils with varying ammonia concentrations [67-69]. The growth of archaea in agricultural soils could, therefore, reasonably serve to remediate soil from intensive agricultural herbicide and fertilization programs worldwide. Microbes, including the documented emergence of archaeal strains in 2007, have been shown to be integral decomposers of residual inputs and biosolids in corn agroecosystems [70-72]. In this process, these archaeal strains are also known to be potent contributors to soil N_2O emissions and may be able to contribute even amid the presence of any nitrification inhibition [73], indicating a widespread genomic adaptability distinct from AOB populations, [74-76]. It has also been suggested that N_2O is emitted as a spontaneous metabolic AO intermediate [73]. Recent research reveals that ammonia-oxidizing archaea are underestimated contributors to N_2O emissions in soil [77], and this discovery is consistent with changing glyphosate dynamics.

N_2O and ADHD

N_2O has been used as an anesthetic agent in health care settings, primarily dentistry. However, there is a growing reappraisal of the compound's potential adverse health effects. We propose that N_2O may be a potential nexus between estimated glyphosate use and increasing healthcare utilization among patients with an all-listed ADHD hospital discharge diagnosis.

Yagiela [78] explains that many of the adverse effects seen with N_2O inhalation are attributable to its reaction with the reduced form of vitamin B12 in the body [79]. Vitamin B12, or cobalamin, is a critical co-factor for methionine synthase, an enzyme necessary for nucleic acid synthesis and methylation reactions. Exposure of infants to N_2O during surgery resulted in a significant increase in plasma levels of homocysteine, due to an impaired ability to convert it to methionine [80].

Methionine synthase inactivity interferes with methylation of the homocysteine subunit of the dopamine receptor 4 [81], leading to an inactivation of dopamine-induced phospholipid methylation (PLM) through dopamine receptor 4 (DR4). NE has been shown to activate

DR4 in rat lateral habenula [82], so it is plausible for there to be excess NE amid DR4 inactivation. Another mode of action of N_2O is thought to be inhibition of N-methyl-D-aspartate (NMDA) receptors [83, 84], which has been implicated in ADHD [85].

Available clinical evidence is suggestive of cognitive impairment in working memory, a core deficiency of ADHD [86], from exposure to trace amounts of N_2O in human male dental and medical students [87] as well as psychosis from significant acute inhalational exposure [88]. We, therefore, are the first to propose that environmental exposure to N_2O may be the mechanism behind the positive association between glyphosate use and all-listed ADHD hospital discharge diagnoses and associated comorbidities during the period 2007 to 2010.

Study Strengths and Limitations

There exist several limitations to the conclusions made from our exploratory investigation. These limitations include, principally, the data itself. We were limited in the number of states we could include due to HCUPNET availability and the availability of regressor data. Furthermore, the USGS data on estimated glyphosate use is normalized according to harvested agricultural acreage within a county and does not overtly account for non-farm use. It is possible that non-farm use of glyphosate and nitrogen fertilizers could account for some of the increase in ADHD hospital discharges, as our data cannot exclude this as a possibility. The USGS report indicates that the ratio of non-farm to total nitrogen input estimates from 1987 to 2006 was higher in several states reporting large increases in all-listed ADHD hospital discharges from 2007 to 2010 (i.e., New Hampshire, Massachusetts, and Rhode Island).

A second limitation of our study is the lack of genetic markers in our model. Genetic risk factors are being studied for ADHD disorders, in addition to other mental disorders. More specifically, LaHoste et al. [89] have implicated genetic polymorphisms within the DR4 gene as a potential contributor to ADHD, and there are many other studies that point to a genetic predisposition for substance and alcohol addiction [90, 91]. In our analysis of national HCUPNET hospitalization data, we find that, as a percentage of total discharges for all mental disorders, discharges for substance/alcohol abuse have actually plateaued, suggesting that genetic polymorphisms contributing to these conditions have remained static in the health care-seeking population. A significant increase in genetic polymorphisms associated with the mental conditions under study is therefore probably not a plausible explanation for the results obtained, although we cannot say unequivocally that this is the case.

A third limitation is that we have not fully considered the interaction of other variables. For example, we have included maize grain in the current study and found that it is a significant predictor of all-listed ADHD hospital discharges even after controlling for glyphosate, suggesting the mechanism could be related to the genetic composition of the corn as discussed earlier, its use as an agricultural residual cover crop, or another interaction yet to be identified. There are other environmental toxicants that have been associated with ADHD as well, and we have not accounted for these variables in our models [92, 93], principally because we were unable to obtain reliable annual state-level estimates for the years of interest. Furthermore, our study period was subject to influence by the worldwide economic recession.

As socioeconomic status has been implicated in ADHD [47], it is possible that our variables of interest (i.e., home price index) were not adequate for capturing the potential effect of the economic recession, and we find this to be a limitation. It should also be mentioned that, while the ADHD population in this study was based on ICD-9 diagnoses, there have been significant diagnostic improvements and greater awareness of mental conditions like ADHD in recent years. It is therefore difficult to know for certain whether such conditions are actually increasing or are the result of major diagnostic advancements and greater public awareness [94]. This is a significant quandary, especially in light of the economic difficulty of the period [95]. Therefore, attempts should be made to replicate these results using a diverse set of patient populations, diagnostic environments, and economic conditions.

A few strengths of our investigation include the use of a fixed effects design, which reduces the likelihood for omitted variable bias. We have also employed health care utilization as an outcome for ADHD, and this decision may successfully screen out "pure" and over-diagnosed cases of ADHD, which may be potential confounders to the true prevalence of the disorder. Additionally, we controlled for the co-occurring nature of other mental disorders with ADHD. To counter the ecological fallacy inherent in studies like ours, we present results that suggest that glyphosate may be acting as an instrumental variable and not as the primary individual exposure [96]. The use of glyphosate may predict health care utilization for ADHD and associated comorbidities *only* through the herbicide's close association with nitrogen fertilizers and via individual exposure to pollutants emanating from fertilizer use, like N_2O emissions.

Conclusions

We have shown that estimated agricultural glyphosate use and total food and industrial use of maize grain are significant lagged predictors of all-listed ADHD hospital discharges in HCUPNET reporting states from 2007 to 2010 after controlling for state fixed effects, strong correlations over time, and other documented associations with ADHD in the literature. The association appears to be most significant in urban counties. Fig. 5 is a global schematic that demonstrates the indirect mechanism that may underlie the increase in healthcare utilization for patients with ADHD seen during our study period.

These significant associations occur amid the absence of estimated glyphosate use predicting resistance events

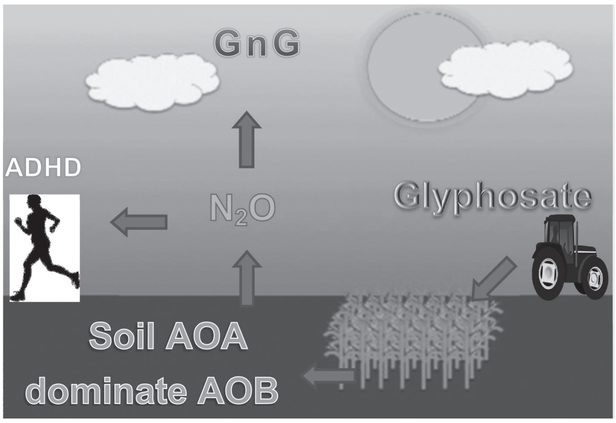

Fig. 5. A global schematic of an indirect mechanism for how glyphosate-based herbicides may be contributing to the rise of ADHD during 2007 to 2010.

and changes in the ability of estimated glyphosate use to predict other mental health discharges. We propose that a patented reformulation in glyphosate could be enhancing both its herbicidal and bactericidal properties, enabling the dominance of the more genetically adaptable archaea in agricultural soils. Maize residuals may also be used as an herbicide control, and this may be contributing to the increase in direct agricultural N_2O emissions – most especially given the changing soil dynamics possibly induced by glyphosate use. Impairment of several physiological mechanisms, including NMDA receptors and methionine synthase, is a likely explanation for how glyphosate may significantly contribute to an increase in healthcare utilization for patients with ADHD. The mechanisms mentioned here require further empirical support to verify these conclusions, and this ought to include a systematic reevaluation of the crucial intermediates involved.

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List of Abbreviations

ADHD	Attention-deficit hyperactivity disorder
USGS	United States Geological Survey
HCUPNET	Healthcare Cost and Utilization Project net
USDA	United States Department of Agriculture
LSDV	Least squares dummy variable
CDC	Centers for Disease Control and Prevention
ASD	Autism spectrum disorder
EPSPS	5-enolpyruvulshikimate-3-phosphate synthase
BT	Bacillus thuringiensis
CYP	Cytochrome P450
N_2O	nitrous oxide
DA	dopamine
NE	norepinephrine
CRD	crop reporting districts
Als	Acetolactate synthase
ACCase	Acetyl-coenzyme A carboxylase
EIA	Energy Information Administration
ISHRW	International Survey on Herbicide Resistant Weeds
ICD-9	International Classification of Disease, 9th Edition
CCS	Clinical Classification Software
BP	Breusch-Pagan
DW	Durbin-Watson
ADF	Augmented Dickey Fuller
Vcov	variance covariance
RUCC	Rural Urban Continuum Code
AOA	ammonia-oxidizing archaea
AO	ammonia oxidation
AOB	ammonia-oxidizing bacteria
PLM	phospholipid methylation
DR4	dopamine receptor 4
NMDA	N-methyl-D-aspartate
GnG	greenhouse gassing

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