Opinion

Glyphosate in northern ecosystems

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Glyphosate is the main nonselective, systemic herbicide used against a wide range of weeds. Its worldwide use has expanded because of extensive use of certain agricultural practices such as no-till cropping, and widespread application of glyphosate-resistant genetically modified crops. Glyphosate has a reputation of being nontoxic to animals and rapidly inactivated in soils. However, recent evidence has cast doubts on its safety. Glyphosate may be retained and transported in soils, and there may be cascading effects on nontarget organisms. These processes may be especially detrimental in northern ecosystems because they are characterized by long biologically inactive winters and short growing seasons. In this opinion article, we discuss the potential ecological, environmental and agricultural risks of intensive glyphosate use in boreal regions.

Glyphosate is weeding the fields

Transition from traditional, low-input agricultural practices to intensive, chemical-driven agriculture has influenced the farming systems in the Western world after World War II. Introduction of a new type of herbicide, glyphosate (e.g., commercial formulation Roundup®) in the 1970s was the beginning of a new era in weed control [1–3]. Glyphosate (see Glossary) verges on a perfect herbicide more than any other herbicide currently on the market, and it is the world's best-selling herbicide due to its affordable price [4].

The mode of herbicidal activity of glyphosate is based on inactivation of an enzyme of the shikimate metabolic pathway (Figure 1). Glyphosate kills nonselectively and efficiently nearly all herbaceous plants. However, it has been regarded harmless to animals and humans, because the shikimate pathway does not occur in animals [4]. Glyphosate should also have limited long-term risks, because it is assumed to be rapidly inactivated in soils by strong sorption to soil particles and fast microbial degradation [5–7]. Thus, glyphosate has been proclaimed safe to nontarget organisms [4,8].

In addition to being cost-effective, glyphosate has also been reputed to cause environmental benefits. It enables no-till cropping that decreases erosion and nutrient leaching without disturbing soil structure and functions [4]. Glyphosate is also used to synchronize and accelerate the ripening of forage cereals. As one of the most efficient herbicides it has not only been used successfully in agriculture but also as a defoliant in forestry [9] and recreational areas, and to control invasive species in the context of conservation [10]. During the past decades transgenic glyphosate resistance has become a standard ingredient in genetically modified crops, which has further increased the use of glyphosate in countries where the use of genetically modified organisms (GMO) is permitted [11,12].

Ideally, glyphosate use should increase profitability of agriculture and decrease load from herbicides more noxious to humans and the environment. However, the actual amounts of glyphosate used are increasing globally and in many areas there is a tendency to use it as the sole herbicide [13]. The proposed insignificance of the impacts of glyphosate on the environment, agriculture and even human health has been recently challenged [14,15]. It is obvious that a thorough understanding of the consequences of widespread glyphosate use in target ecosystems and its ramifications for surrounding areas is lacking. In this opinion article, we focus on biological consequences of intensive glyphosate use especially in northern ecosystems in which glyphosate may persist longer because of the prevalent soil types and climatic conditions, and thus have unforeseen consequences on nontarget organisms. We propose that these consequences have largely been underestimated due to the limited study conditions.

Degradation of glyphosate in northern ecosystems

It is widely believed that glyphosate degrades in 2 weeks, and has low accumulation and drift in nature [4,16]. However, this conventional view may be premature, or only applicable to certain environments, because most literature on the biological impacts of glyphosate is based either on laboratory bioassays or short-term field studies, conducted primarily in agro-environments and limited climatic conditions, mainly in Central Europe and the USA. For example, studies on herbicide residues in boreal environments have demonstrated that glyphosate and the main

Glossary

AMPA: 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid, the main degradation product of glyphosate in soil. AMPA is assumed to be as toxic as glyphosate to nontarget organisms.
EPSPS: 5-enolpyruvylshikimate-3-phosphate synthase, the molecular target site of glyphosate in the shikimate pathway
Glyphosate: *N*-(phosphonomethyl)-glycine, a broad spectrum systemic herbicide used to kill weeds.
Nontarget plants: crop plants not intended to be treated with herbicides.
No-till cropping system: growing crops without tilling the soil and thus reducing erosion and nutrient leaching. It requires herbicide application to prevent competition of weeds with the crop plants.
Shikimate pathway: biosynthetic sequence in plants microbes to produce the aromatic amino acids phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp).
Silviculture: practice of controlling the establishment, growth, composition, health and quality of forests.
Target plants: weeds that are treated with herbicide to prevent competition with crop plants.

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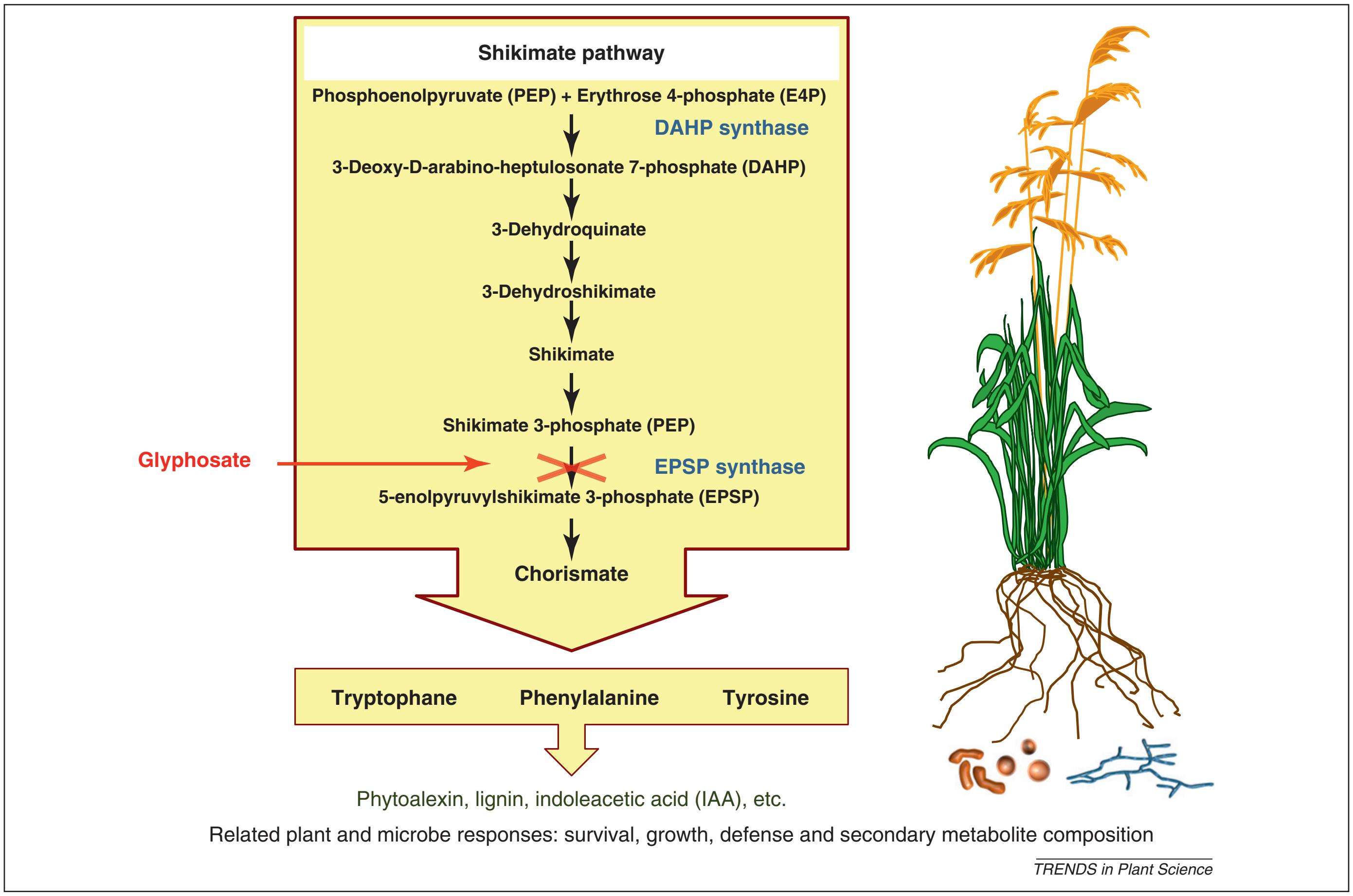

Figure 1. Glyphosate inhibits the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimate acid pathway, thereby interfering in the production of proteins and other molecules that require tryptophan, phenylalanine or tyrosine as precursors. Some of the blocked molecules act as growth promoters (e.g., indoleacetic acid, IAA) or defense metabolites (e.g., tannins, anthocyanins, flavonoids and lignin) for the plants [67]. The shikimate pathway can be found in plants and microbes.

metabolite of glyphosate degradation, 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid (AMPA), can be traced from soils even years after the last spraying [15,17,18].

Glyphosate is applied to green leaves of target plants, where it moves throughout the plant especially to apical meristems and roots, and thus comes into contact with root and soil associated microbes (Figure 2). Because glyphosate is not degraded within the plant, large root systems of some weeds are transporting glyphosate to deep soil layers where microbe activity is relatively low [19,20].

The persistence and transport of glyphosate in soil is dependent on soil composition, climatic conditions and microbial activity [1,21], as well as agricultural management [22]. Undegraded glyphosate is almost instantaneously inactivated by sorption to soil particles reducing its transport in the soil matrix or leaching in soluble form. For example, strong cations (Fe, Al) in soil and in water react with glyphosate to produce compounds that degrade very slowly. This might partly explain why farmers have long known that the same amount of glyphosate is less effective, when glyphosate is diluted in very hard water with high mineral content. In boreal areas, soils tend to have low pH, which helps glyphosate to be sorbed to mineral particles [3].

Mere chemical processes are unable to break the C–P bonds of the glyphosate molecule. Instead, free glyphosate in soil is degraded to CO_2 and NH_3 by microbes, mainly bacteria such as *Pseudomonas* [3], whose activity is affected, for example, by temperature, acidity and moisture [20]. Soil microbes in general are poorly known, and even less is known of their variability, growth rate and function in northern ecosystems. The microbial species [23] and even strains [24] differ in their efficiency and mode of degrading glyphosate. Still it seems clear that in northern climatic conditions strong seasonality limits the time period of peak activity of glyphosate degradation to summer months. Furthermore, there is pressure to give glyphosate treatments late in season to accelerate ripening and kill the weeds before seed set. Thus, the half-life time of glyphosate may be much longer in northern ecosystems than generally presumed [3,22].

Glyphosate and phosphorus competing in soil

Phosphate is playing a particularly important role in determining the availability of glyphosate to organisms in soils because of shared adsorption mechanisms and sites for phosphorus and glyphosate in soils [21,25]. Although depending on soil characteristics and other environmental conditions, phosphorus can outcompete glyphosate for soil sorption sites. For example, soil pH is the most important single factor for glyphosate sorption, which is negatively correlated with acidity [3], whereas phosphorus adsorption in soil is not strongly determined

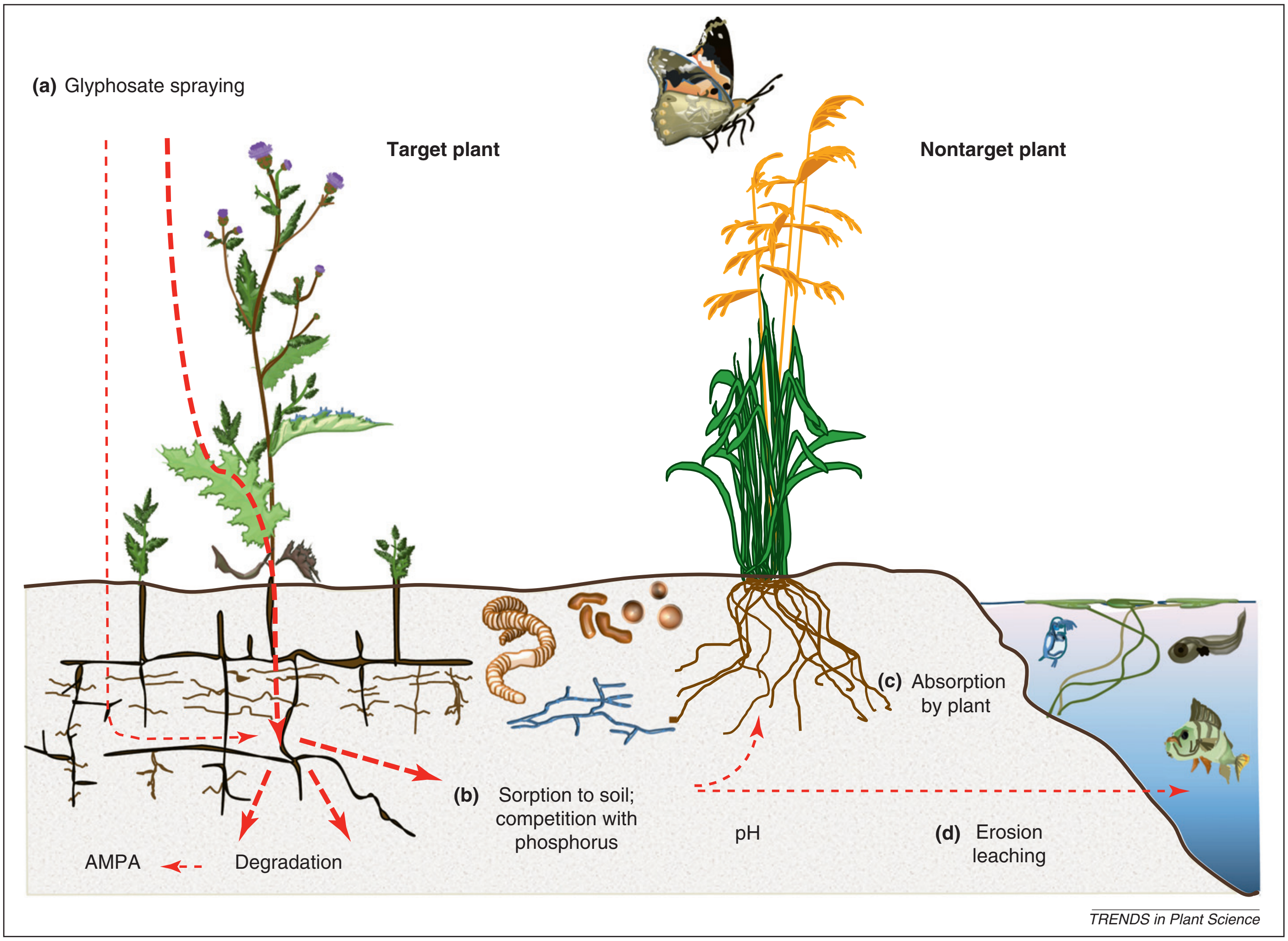

Figure 2. Glyphosate accumulation and transport in the field. Red arrows indicate transport of glyphosate. **(a)** Part of the applied glyphosate may end up directly at the soil surface. In living plants, glyphosate is first absorbed by foliage and then translocated throughout the plant including shoots and roots via the phloem. Thus, glyphosate can be traced in all plant parts. **(b)** From the roots glyphosate can be exuded into soil or remain in withering root parts and then be consumed by animals living in the soil (e.g., earthworms) feeding on dead plant material, degraded by soil microbes, or adsorbed to the soil particles. Part of the glyphosate may be degraded to 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid (AMPA; the main metabolite of glyphosate). Reactions in soils are affected by soil type and mineralogy, and by competition on sorption surfaces (e.g., by inorganic phosphorus). **(c)** Residues of glyphosate may be taken up by nontarget plants influencing their growth and resistance to pathogens and herbivores. A residual part of glyphosate may end up in animal feed (and finally be part of manure that is spread in fields) or find its way into human consumption. **(d)** Erosion in soil particles and leaching of surface water is transporting glyphosate remains to aquatic systems.

by soil pH [1,21]. Furthermore, different microbial species have different mechanisms of glyphosate degradation (either via AMPA or sarcosine), and ample presence of glyphosate and the presence or absence of phosphorus in the environment causes selection pressure for microbial flora [26,27]. This complexity of the degradation and adsorption calls for a deeper understanding of soil chemistry and emphasizes the importance of long-term studies on accumulation and translocation of glyphosate and its main metabolites.

These questions have special importance in agro-ecosystems in northern latitudes where biological activity is restricted by seasonality. For example, European arable soils have been fertilized with phosphate for decades in excess of what is absorbed and removed by the crops [28,29]. Therefore, phosphate has accumulated in the soils, leading to a lower capacity to adsorb phosphate and glyphosate. However, in areas where glyphosate has previously been used, addition of phosphate may lead to remobilization of glyphosate residues in soils [25]. In contrast to arable soils, the microbes probably degrade glyphosate to gain phosphorus which is, other than nitrogen, the growth-limiting nutrient in boreal forests. Corresponding conditions, characterized by similar seasonal restrictions such as low winter temperatures and/or frost and frequent rains during the growing season, are also widely detected in northern Eurasia and North America. Therefore, thorough understanding of soil chemistry under the prevailing climatic conditions is needed to be able to foresee the consequences of glyphosate use in target-ecosystems and surrounding catchment areas.

Biological consequences

Accumulating evidence has revealed that glyphosate use may shape biodiversity and therefore ecosystem functions and services. In addition to target plants (weeds), glyphosate interactions extend to nontarget plants (crops and wild plants out of agronomic areas) and other organisms (microbes and animals) in both terrestrial and aquatic environments.

Glyphosate has been observed to accumulate in plant roots from where it is gradually released into the rhizosphere [19,30]. Because glyphosate blocks the shikimic acid pathway not only in plants but also in some fungi and bacteria (Figure 1), it affects the microbial activity and modifies microbial community in the soil [2,6,31,32]. If the soil microbial communities responsible for decomposing organic material are negatively affected by herbicide treatments, decomposing rate of biomass and nutrient cycling is potentially altered when glyphosate is applied. In addition to agriculture, glyphosate is the preferred herbicide in boreal forests due to its mild effects on conifers [6]. For example, in Canadian forest practices glyphosate is commonly applied aerially from small aircrafts [33]. The use of glyphosate in silviculture has been challenged, but economic benefits outcompete the environmental aspects [34,35]. Furthermore, repeated glyphosate applications may decrease the number and diversity of microbes capable of degrading glyphosate and thus increasing the glyphosate remaining in the soil and its risk of leaching into nearby waterways.

Although glyphosate has been shown to be able to control nearly all weed species, the timing and frequency of glyphosate use have caused shifts in weed populations towards annual broad-leaved species and very deep-rooted species [36]. In addition, the repetitious use of the same herbicide over an extended period has caused documented cases of herbicide resistance in weeds [12,37,38]. The genetic basis of glyphosate resistance can occur through multiple pathways [12]. It is likely to be that resistance has appeared and is evolving independently in different populations [39]. The mode of action of glyphosate is so unique that weed resistance development was regarded most unlikely, and it took 20 years before the first glyphosate-resistant weeds appeared. During the past decades, glyphosate has become the backbone of no-till agriculture with almost 90% of all transgenic crops worldwide being glyphosate-resistant and in many areas hardly any other herbicides are used [4]. This causes a very strong selection pressure for increasing glyphosate resistance in weeds. Presently, glyphosate resistance has been documented in more than 20 weed species [12].

In addition to direct negative effects on plants, extended use of glyphosate has been suggested in some cases to benefit disease causing microbes. Glyphosate may be among the most important agronomic factors increasing plant disease incidence in wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) crops [40,41]. Glyphosate use in silviculture has also been connected to blue stain fungi in poplar [42]. Sublethal doses of glyphosate to plants can decrease plant resistance to pathogens and herbivores by reducing their secondary metabolite production (Figure 1) and decreasing uptake of micronutrients (e.g., Mn) and subsequent development of deficiency symptoms in non-target crops [32,43–46].

Toxic effects of glyphosate, its main metabolite AMPA and surfactants used in herbicide formulations are documented in several bacteria and fungi [6,31], as well as in both invertebrates and vertebrates in terrestrial and aquatic ecosystems [47–49]. For example, exposure to glyphosate decreases the number of surviving tadpoles, toads and frogs in nature [50,51], and arthropods have been observed to change their behavior and long-term survival [52].

Owing to high water solubility of glyphosate-based formulations and their extensive use in the agro-environment, these herbicides are a concern to water ecosystems. Negative effects of herbicides in freshwater systems have been shown as reduced reproduction of frogs and fish [53–57], decreased survival of algae [58], and increased populations of toxic, bloom-forming cyanobacteria which are capable of using the glyphosate as a phosphorus source [59].

Finally, effects on humans should not be ignored, because we may be exposed to glyphosate when handling the herbicide or herbicide-treated plant material, or if glyphosate enters the food chain via crop plants exposed to glyphosate residues from soil, or via contaminated drinking water. It is noteworthy that the Roundup® dilution levels used in relevant studies were far below agricultural recommendations (i.e., direct contact in spraying), but corresponded to low levels of residues found in food and in animal feed. Glyphosate residues are expensive to detect, and routine monitoring is rarely conducted. Laboratory tests have shown that glyphosate-treated human umbilical, embryonic and placental cells suffer from apoptosis, necrosis and other toxic effects [14,47]. However, rigorous studies on possible effects of glyphosate residues in human diets, for example, through glyphosate-treated crops or contaminated drinking water, are lacking because glyphosate is assumed to degrade quickly in soil.

Environmental concerns

Increasing evidence of accumulation and transport [3,7,15] and interactions with target plants (weeds) [12], nontarget plants (crops) [60] and other organisms (humans, animals and microbes) [14,61] (Figure 2) have raised serious concerns about the continuing and increasing use of glyphosate as the main weed management strategy. It is noteworthy that in addition to glyphosate itself, risks of herbicides might be associated with the main metabolite of glyphosate degradation, AMPA, or surfactants, which might be more toxic than the glyphosate itself [47,62].

Glyphosate is not entirely and immediately degraded and immobilized in soils, as previously suggested. Furthermore, effects of glyphosate and AMPA on biological interactions can be complex and multidirectional. For example, studies on glyphosate interactions with soil microorganisms have demonstrated that although glyphosate is metabolized by some microbes, it is also toxic to several bacteria and fungi [6,31], and increases growth of some microbes [63]. Recent evidence suggests that the decrease of soil microbes or changes in their community composition due to repeated glyphosate applications slows glyphosate degradation in soil [64]. Highly repeated applications on no-till soils may lead to accumulation of glyphosate in the surface soil and increased risk of transport through eroded soil particles by surface runoff, or through earthworm burrows and cracks to subsurface drainage systems.

Environmental risks of glyphosate use are likely to be pronounced in northern ecosystems if environments suitable for agriculture and silviculture will shift to higher latitudes as recently suggested [65,66]. Abundant reservoirs of freshwater, low pH in soils and soil types make these ecosystems particularly vulnerable.

Concluding remarks

Effects of glyphosate on nontarget organisms, trophic interactions on crops and weeds, and cascading effects on food webs are complicated and presumably often difficult to perceive. A better understanding of the uptake mechanisms, degradation pathways and overall actions of glyphosate in the ecosystem is essential in developing sustainable weed management strategies in agriculture and forestry. This requires multidisciplinary long-term field studies combining expertise from physiology and ecology. We propose that the effects on nontarget organisms are likely to be more pronounced and long lasting in northern ecosystems because of increasing use of herbicides in forestry and agriculture, as well as the cold climate comprising a challenge to glyphosate degradation in the soil. The global issues are analogous to those of excessive use of antibiotics: we must avoid the loss of the long-term efficacy of the world's most important herbicide. At the same time we need to be cautious before exploiting the potential new global agricultural areas and freshwater reservoirs in northern latitudes.

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