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A meta-analysis and review of plant-growth response to humic substances: practical implications for agriculture.

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Abstract

The breakdown products of plant and animal remains, extracted in an alkaline solution, are commonly referred to as humic substances (HS). They can be extracted from a wide variety of sources, including sub-bituminous coals, lignites (brown coals), peat, soil, composts and raw organic wastes. The application of HS to plants has the potential to improve plant growth, but the extent of plant-growth promotion is inconsistent and relatively unpredictable when compared to inorganic fertilisers. The goal of this review was to determine the magnitude and likelihood of plant growth response to HS and to rank the factors contributing to positive growth promotion. These factors included the source of the HS, the environmental growing conditions, the type of plant being treated and the manner of HS application. Literature reports of exogenously applied HS-plant interactions were collated and quantitatively analysed using meta-analytic and regression tree techniques. Overall, random effects meta-analysis estimated shoot dry weight increases of 22±4% and root dry weight increases of 21±6% in response to HS application. Nevertheless, actual responses varied considerably and were mainly influenced by the source of the HS applied, the rate of HS application and to a lesser extent, plant type and growing conditions. HS from compost sources significantly outperformed lignite and peat-derived HS in terms of growth promotion, whilst HS application rate non-linearly moderated the growth response under different circumstances. Our results demonstrate the difficulty in generalising recommendations for the use of HS in agriculture; however some specific suggestions for maximising the efficacy of HS under certain conditions are offered. We also outline some recent developments in the use of HS as synergists for improving fertiliser use efficiency and the activity of microbial inoculants. Finally, we identify a number of research gaps, which, when addressed, should clarify how, when and where HS can be best applied for the greatest benefit.

1. Introduction

Humic substances (HS) are a category of naturally occurring organic compounds that arise from the decomposition and transformation of plant, animal and microbial residues (MacCarthy, 2001). They are a natural component of practically all soils, but levels vary and there is considerable evidence that modern agriculture involving practices such as soil tillage has resulted in their decline (Novotny et al., 1999; Shepherd et al., 2001). The loss of humic material, together with overall reductions in soil organic matter, is of concern because they play important roles in maintaining key soil functions and plant productivity (Lal, 2004; Sparling et al., 2006). Consequently, there is interest in the application of HS-based amendments to agricultural systems in order to reverse this trend (Piccolo and Mbagwu, 1997; Quilty and Cattle, 2011).

Humic substances are chemically complex with no clearly defined chemical structure, although generalized models have been proposed (Bruccoleri et al., 2001). While traditionally viewed as complex macromolecules, they have more recently been described as mixtures of smaller molecules, containing aromatic rings, aliphatic chains and ionisable functional groups that interact with each other to form aggregated colloids (Piccolo, 2001; Pinton et al., 2009; Sutton and Sposito, 2005). There is significant evidence that the exogenous application of HS can help to improve soil fertility, primarily through their complex chemistry which facilitates interactions with a variety of mineral and non-mineral organic soil components. Some of the documented benefits of soil amendment with HS include improved soil aggregation and structure, increased pH buffering and cation exchange capacity, increased water retention capacity, increased bioavailability of immobile nutrients (such as P, Fe and Zn), and decreased toxicity of aluminium and heavy metals (Chen et al., 2004a; Imbufe et al., 2005; Peiris et al., 2002; Piccolo et al., 1996; Piccolo and Mbagwu, 1989; Piccolo et al., 1997; Tan and Binger, 1986).

As well as indirectly influencing plant productivity through modification of soil characteristics, HS can also directly impact on physical and metabolic plant processes. A recent review by Muscolo et al. (2013) reviews evidence for the hormone-like effects of HS and how these relate to the chemical structural features of these materials. The authors highlight a predominance of auxin-like effects and that non-lignin structures are the principal contributors. These effects can be elicited through an interaction with either roots or shoots. For example, hormonal-like responses on plant roots were demonstrated by Trevisan et al. (2010) and HS may also stimulate H^+-ATPase and ion transporter activity in the root plasma membrane (Mora et al., 2010; Pinton et al., 1997; Pinton et al., 2009). Both these effects can enhance nutrient acquisition, the former through increased soil exploration, and the latter by accelerating nutrient uptake. These effects appear to be especially prominent for cases involving HS derived from compost and vermicomposts, which may contain auxin-related compounds (Muscolo et al., 1999; Quaggiotti et al., 2004), including indole-acetic acid derivatives and other low-molecular weight organic acids (Russell et al., 2006). In contrast, effects on leaf function have been less well documented and appear somewhat contradictory (Nardi et al., 2002). Foliar application of HS may increase leaf chlorophyll concentration (Sladký, 1959), but it is also recognized that HS contain a range of functional groups which are able to interfere with photosynthesis (Pflugmacher et al., 2006). Foliar applications have also been shown to influence transpiration, though the mechanism is unclear and both increases and decreases in water loss and leaf gas exchange have been observed.

Despite numerous publications on the potential positive effects of HS on plant growth and productivity over more than five decades (Billingham, 2012; Chen et al., 2004b; Quilty and Cattle, 2011) and substantial interest in their potential for improving nutrient-use efficiency and contributing to C sequestration in the soil, the use of commercial products containing HS in agriculture varies and there is scepticism about their effectiveness (Billingham, 2012). Part of the reason for this is no doubt related to the wide range in physico-chemical properties of HS, which vary according to the method of extraction and the environmental matrix from which they are sourced. HS are formed under a variety of environmental conditions and are, therefore, highly heterogeneous and structurally difficult to define (Senesi, 1994). Commercial products often contain mixtures of humic materials and added plant nutrients; hence the cause of any observed beneficial effect cannot be easily attributed to the HS themselves. In addition, the recommended rates of application of commercial products are generally very small in relation to the natural levels of HS present in the soil. As a consequence, the effect of a HS product is substantially less predictable than other plant or soil amendments of a known chemical structure, such as inorganic fertilisers or synthetic organics including pesticides and growth regulators. Moreover, because of the multiple chemical functional groups of HS, a particular HS product may behave completely differently under different environmental conditions, or when applied to different plant species. Finally, as with many chemical fertilizers, the timing, location and rate of application will play a crucial role in determining whether beneficial or harmful effects will evolve and whether or not any beneficial effects are economically worthwhile. This is particularly important because recent publications have pointed out potential negative effects and have questioned the economic viability of applying HS for improved crop production (Asli and Neumann, 2010; de Santiago et al., 2010; Hartz and Bottoms, 2010).

In light of the potential benefits of HS, together with their inconsistent performance under field conditions, we sought to improve the understanding of the effects of HS on plant growth by conducting a meta-analysis of published literature. More specifically, our objectives were (i) to quantify the magnitude and likelihood of plant growth promotion, in terms of shoot and root biomass, resulting from HS application, (ii) to determine the influence of environmental conditions, plant type, HS properties, and the manner of application on plant growth response to HS, (iii) to identify gaps in our understanding of the interaction of HS with plants, and (iv) to provide some general recommendations for the practical use of HS in agronomic systems and suggestions for future work.

2. Meta-analysis

2.1 Methods

2.1.1 Literature search and refinement

We conducted a search of the databases Scopus and ISI Web of Science using a combination of search terms including ‘humic’ AND ‘plant’ AND ‘effect’ AND ‘growth OR yield’. This search was designed to provide an un-biased selection of potential studies, rather than act as an exhaustive search for all studies in this area. The search yielded 390 papers, the abstracts of which were screened in the first instance to determine if the experiments conducted involved the application of HS to plants. Unsuitable abstracts (no plants grown or no HS applied: 185 papers), non-research articles (6 papers) and publications in languages other than English (19 papers), were not reviewed further. The full text of all remaining papers were sought and scrutinized to determine if a measure of plant shoot (SDW) or root (RDW) dry weight was reported for both a HS treatment and a suitable untreated control. Papers not fulfilling this minimum requirement were also excluded from our analysis (99 papers). The full reference list including rejected papers is available on request or on our website (soilecology.org), and a list of accepted papers is given in Appendix 1 and Appendix 2. From a total of 390 papers originally found, 81 were retained for the meta-analysis; with 57 studies presenting data on SDW and 39 studies reporting RDW. This provided over 700 data points on which to base our analysis, which can be updated and expanded in future as research progresses. It is important to note that few of the retrieved studies report results from statistically rigorous field trials testing plant growth responses to HS through to crop maturity. We would like to emphasize that our meta-analysis therefore reflects this limitation, but nevertheless provides important information about trends in plant growth response and how they might be manipulated for maximum agronomic benefit.

2.1.2 Response and moderator (explanatory) variables

The focus of many investigations is on either shoot or root responses to HS, but not both; consequently we assessed SDW and root dry weight RDW as separate response variables. We were also interested to examine if HS affect both root and shoot biomass in a similar manner, or whether growth effects are biased toward either plant organ under different circumstances. In order to test our hypotheses we used the data available in the papers included in our analysis to identify a set of continuous and categorical groups that we predicted would influence the responsiveness of plants to HS applications. These groups fell under four broad areas: environmental conditions; plant type; HS properties; and the method of HS application.

2.1.2.1 Environmental conditions

Originally we attempted to populate a data matrix containing quantitative data of experimental growth conditions, including pH, EC, nutrient availability and temperature; however, full data sets were rare, and we did not further pursue this avenue of investigation. Instead, we created two proxy categories based on data that were routinely reported: growth media and stress conditions. Growth media contained three levels: hydroponic culture, soil culture or hybrid culture. Hybrid culture entailed the growth of plants on a solid, but relatively inert media (for example sand, vermiculite, perlite or peat) and regular fertilisation with nutrient solution. The stress conditions category was also designated into one of three levels: no stress, moderate stress or high stress. No stress included studies that did not explicitly state stress as an investigation factor, or did not include treatments (other than HS application) that reduced growth to less than 90% of non-treated controls. Moderate and high stress involved treatments (additional to HS application) that reduced growth by 10-50% or >50% as compared with non-stress controls, respectively.

2.1.2.2 Plant type

The plant species used in each study was recorded and subsequently categorised into three levels: monocotyledonous plants, dicotyledonous herbs and woody perennials.

2.1.2.3 Humic substance properties

To characterise the HS used, we initially tried to obtain quantitative chemical data on the composition of HS used in each study, such as percentage C, H, N and O; molecular weight range distribution; and carbon functional group composition as analysed by nuclear magnetic resonance spectroscopy (NMR). Unfortunately, such data were sparse. As an alternative, we created a sub-category based on the source of the humic acids, which included brown coal, peat, soil, compost (green waste), compost (manure) and unreported. The level 'brown coal' included HS extracted from lignite, leonardite and sub-bitumous coals. Although many papers used commercial HS, these were usually identified by trade name or manufacturer and could therefore be traced to the original source. Composts included both vermicomposts and traditional composts.

2.1.2.4 Method of application

Two sub-categories were created to characterise the method of HS application. The first included the 'site' of application as foliar-applied, root-applied, combined foliar-root application or soil-application. Root-application generally involved addition of the HS into the growing medium. HS applied to seed were designated as 'combined' application, our rationale being that both the roots and shoots come into contact with the HS on germination. The second moderator within this category was a continuous variable that specified the rate of HS application. All rates were converted into mg of HS per kg of growing medium. In the case where rates were reported as mass of HS per volume of growing medium and bulk density was not given, a bulk density of 1.0 g cm^{-3} was assumed. In the case where rates were reported as mass of HS per unit area, we assumed passive incorporation to a depth of 10 mm, and again, a bulk density of 1.0 if not otherwise reported.

2.1.3 Statistical analyses

Response ratios to HS treatment were calculated for SDW and RDW, such that,

$$L = \mathrm{Ln}(DW_{HS}/DW_{C})$$

Where DW_{HS} is the dry weight of shoot or root biomass of plants treated with HS, and DW_{C} is the dry weight of the non-treated control grown under the same conditions. The variance of the response ratio was calculated according to Hedges et al. (1999) using the standard error and number of replicates reported for each individual study. Where standard errors were not presented or could not be calculated, we assumed a standard error of 10% of the mean (Gattinger et al., 2012; Luo et al., 2006). Response ratios were analysed using the 'metafor' package (Viechtbauer, 2010) within the statistical program R (R Development Core Team, 2005). The 'metafor' package provides functions for fitting both fixed- and random-effects models to observed outcome measures, with or without the inclusion of moderator variables (study-level covariates). For our purpose, the SDW or RDW response was taken as the observed outcome measure, and the variables growth media, stress condition, plant type, HS source, application site and application rate were designated as moderators. The overall heterogeneity was initially assessed by excluding all moderator variables, and each moderator was subsequently tested one-by-one as a sole covariate, in order to ascertain its individual power to explain the observed heterogeneity. All models were run using the restricted maximum-likelihood estimator function. Publication bias was assessed by creating funnel plots (Egger et al., 1997) and assessing asymmetry in the data by conducting a meta-analytic regression test using variance as a predictor in the 'metafor' function regtest.rma (Viechtbauer, 2010).

Although mixed-effect modelling in this framework is useful for combining multiple studies and estimating aggregate effects of covariates, it lacks the capability to model non-linear functions and is not very efficient for modelling interactions between variables, both of which are common occurrences in environmental systems. In order to further explore the complex nature of HS effects on plant growth, we therefore also conducted classification and regression tree (CART) modelling (De'ath and Fabricius, 2000). This non-parametric approach repeatedly splits heterogeneous data into increasingly homogeneous subsets. It is commonly used to establish prediction criteria based on a number of explanatory variables, but can also be used to rank the importance of these explanatory variables in describing the overall heterogeneity of a data set (De'ath and Fabricius, 2000). Depending on splitting criteria, a number of different CARTs can be produced from the same data set, with differing bias and predictive power. To overcome the issues inherent in constructing a singular CART, we performed a boosted regression tree (BRT) using the R package 'gbm' (Ridgeway, 2013) combined with the 'rt' vignette (Elith and Leathwick, 2013; Elith et al., 2008). Boosting grows the suite of trees by sequentially modelling the residuals throughout all parts of the data space, including those for atypical observations that depart from the dominant patterns explained by the initial trees (Elith et al., 2008). Weakly predictive trees are aggregated to create an improved model, thereby reducing both bias (through forward stagewise fitting) and variance (through model averaging).

Using the output from the regression tree analysis, we partitioned the data sets according to the two most influential explanatory factors, and plotted the growth response against the rate of HS application. Inferences were made by fitting linear models to log-transformed data.

2.2 Results

2.2.1 Data quality and aggregate effect of HS on plant growth

Case diagnostics performed using the 'influence' function of the metafor package identified 12 outlier data points in the SDW data set and four outliers in the RDW data set that exerted considerable influence on the random effects model fit; these data points were therefore excluded from the model. The revised random effects model predicted that HS application significantly (Table 1) increases both SDW and RDW by 19±3 % and 20±4%, respectively. Publication bias was not detected by regression tests of funnel plot asymmetry for either the SDW (p=0.96) or the RDW (p= 0.51) data set.

Subsequent inclusion of moderator variables into the model showed that the shoot growth response was not significantly influenced by the growth media or the application site, but was significantly affected by the source of HS used, stressful growing conditions, the type of plant being treated and the rate of HS applied (Table 1). Of the HS source categories, only peat-derived HS did not significantly affect shoot biomass accumulation (4±12%) (Figure 1). Brown coal derived HS increased SDW response (12±4%), but was less effective than HS extracted from green waste compost (29±8%), manure compost (28±8%) and soil (25±8%). Plants were significantly more likely to increase shoot growth in response to HS application under highly stressful conditions (28 ±6%) than non-stressful conditions (18±3%). Also, woody perennials did not show any significant shoot growth promotion in response to HS application.

In contrast to shoot growth, the effect of HS on root growth was not significantly influenced by stress or application rate, but the source of the HS still moderated the growth response in a similar fashion to that of shoots (Table 1). Under these circumstances, both peat- and brown coal-derived HS did not affect root growth, but all other HS promoted plant root growth by 12-40% (Figure 2). The root growth of woody perennials was similarly not significantly affected by HS application, and although the growth media moderated the root growth response (Table 1), there were no significant differences between the different growth media (Figure 2).

2.2.2 Factors influencing HS efficacy

To further investigate the source for variability in plant growth response to HS, a boosted regression tree model (BRT) was constructed and analysed. The optimised BRT was superior to the mixed-model meta-analysis in terms of model fit to both SDW and RDW. The BRT revealed that application rate, HS source and plant type were the most important factors regulating on HS impact on shoot and root growth; this was in agreement with the results of the mixed effect model (Figure 3). In comparison, application rate, HS source and stress conditions were most important for root growth. The growth media used and the location of application played less of a role in influencing HS efficacy than the other variables.

The distributions of the modelled data emphasized the variability in response of plant growth to HS application (Figure 4 and Figure 5). As in the mixed model, brown coal- and peat-derived HS did not promote plant growth as strongly as other HS, and woody perennials generally responded negatively to HS application. Trends in other explanatory variables were not readily apparent, suggesting more complex interactions between variables were responsible for explaining the observed heterogeneity of shoot and root growth response.

2.2.3 Factor interactions

Interactions between HS source and application rate were found to be important in explaining the variation in both shoot and root growth response to HS. An interaction between plant type and application rate was also apparent for shoot growth, whilst an interaction between HS source and stress conditions was the most important pairwise interaction involved in root growth response (Table 2).

To further investigate the interactive effects revealed in our analysis, we re-plotted both sets of response data according to the interactions between the three most important explanatory variables; in the case of shoots this was application rate, HS source, and plant type and for roots this was application rate, HS source and stress conditions. Whereas increasing rates of green waste compost HS application to both monocots and dicots was positively related to shoot growth over untreated control plants, the application of soil-derived HS appeared to stimulate plant shoot growth more effectively at lower application rates (Figure 6). Furthermore, higher rates of brown coal and peat derived HS appeared to inhibit shoot growth in woody perennials relative to untreated controls, but the application rate of these HS did not affect the shoot growth of monocots and dicots in any consistent fashion.

With regard to root biomass response (Figure 7), increasing rates of brown-coal derived HS were negatively related to root growth under conditions of stress, but did not consistently affect growth under non-stress conditions. The opposite occurred with soil-derived HS, with a positive root growth response to increased application rates under high stress conditions. However, as with brown-coal derived HS, inconsistent effects were observed under low and non-stress conditions.

3. Plant growth response to Humic Substances: moderating factors

3.1 General plant growth response

Humic substances are becoming increasingly available as commercial supplements for crop improvement, but growth effects can be positive or negative and difficult to predict (Quilty and Cattle, 2011). In considering a wide range of published studies, we found that HS generally increase shoot and root growth by 15-25%, but high variation increases risk to farmers. For example, approximately half of the studies on SDW response and one-third of RDW studies failed to increase growth by more than 5%, which we consider to be agronomically significant. Thus there is a strong need to improve consistency and predictability of the growth response.

3.2 Application rate

Regression tree-modelling showed the rate of HS application and its interaction with other factors as important predictors of growth promotion by HS. This is in contrast to the *linear* mixed effect modelling, which suggested only a minor significance to shoot dry weight. The contradiction between the two models implies that although the application rate has a strong influence on the growth of plants receiving HS, the response is non-linear. Biological responses to increasing concentrations of HS are often best-described by quadratic functions, whereby an optimum concentration is identified after which the response declines or inhibition occurs (Chen and Aviad, 1990; Dobbss et al., 2010; Liu et al., 1998; Schluckebier and Martin, 1997). Although quadratic functions may hold over concentration ranges covering an order or two of magnitude, there is some evidence that other functions (e.g. cubic) can sometimes be more appropriate (Liu et al., 1998). In these situations, where positive-negative-positive responses are observed, we speculate HS operate through different mechanisms that only become pronounced at particular concentrations. Chen et al. (2004a) suggest that in hydroponic studies, this occurs because of increasing then decreasing bioavailability of micronutrients brought about by HS-micronutrient complex stability. In soil environments, similar chemical and biological processes may occur at lower rates, whilst physical effects might begin to dominate at higher rates. Importantly, interactions between the application rate and other factors means that a particular response curve generated under one condition is unlikely to be transferable to other conditions. This was illustrated by Dobbss et al. (2010), who used a quadratic function to describe plant root branching stimulated by different concentrations of HS derived from vermicompost, but found that the optimal dose varied between the HS used and also the plants to which they were applied.

3.3 Humic substance properties

Plant growth responses are strongly affected by the type of HS applied. The importance of the source of HS was discussed by Chen et al. (2004a), who attributed the variability in plant growth response to the variability in HS used before the introduction of standardized extraction procedures by the International Humic Substances Society. Our study and analysis of the literature suggests that compost- and soil-derived HS have a greater positive effect than brown coal and peat-derived HS. Such an effect is likely to be related to the chemical structure of HS derived from each source, and possibly also related to co-extracted mineral nutrients remaining in HS formulations.

With regard to the chemical structure of HS, we hypothesise that the N content of the compost and soil-derived HS, which is generally higher than that found in brown coal and peat-derived HS (e.g. (Simpson et al., 2003)), could be a strong driver of growth via a number of mechanisms. First, mineralization of HS can liberate plant-available N (and possibly other nutrients, such as P) to stimulate plant growth (Alvarez and Steinbach, 2011; Valdrighi et al., 1996). Amide (N-containing) functional groups of HS become quickly depleted in soils (Tatzber et al., 2009a), with initial decomposition half-lives of HS derived from fresh animal manures being as rapid as 2-4 months (Tatzber et al., 2009b). Because compost and soil derived HS are generally at a lower stage of humification than brown coal and peat HS, their decomposition by biological activity is likely to be faster. The fact that green waste compost-HS application leads to increased plant productivity in a dose-dependent manner (Figure 6) supports a direct N-fertilization hypothesis: for example, 50% mineralization of compost HS containing 5% N applied at 1000 mg kg^{-1} would provide approximately 25 kg mineral N per ha in a 0.01 m layer. Indeed, in the study conducted by (Valdrighi et al., 1996), compost-derived HS only significantly improved plant growth at rates $\geq$ 1000 mg kg^{-1}. In comparison, more biologically stable BC-derived HS, containing only 1-2% N and usually applied at lower rates, is unlikely to contribute to the nitrogen nutrition of plants to any appreciable extent.

Although this explanation is appropriate for the case for high-N containing HS applied at high rates, it does not account for their performance when applied at lower rates. Examination of the rate-dependent effects of soil-derived HS (Figure 6) shows a positive growth response between approximately 25-750 mg kg^{-1} that declines at higher rates. Low rates of most HS should not contribute enough N to explain observed growth increases. An alternative, or complementary, mechanism may involve direct stimulation of plant growth through hormone-like activity at lower HS concentrations. Hormone-like activity of HS has been linked to N-containing compounds, including indoles such as auxins (Nardi et al., 2000), and polyamines (Young and Chen, 1997). More recently, Canellas et al. (2012) showed that the induction of lateral roots in plants by HS is positively related to the hydrophilicity of the HS, especially the O-alkyl and methoxyl/N-alkyl chemical functional groups identified by NMR. Less-humified HS that contain more polar N- and carboxyl-functional groups also exhibit a greater ability to chelate micronutrient elements, such as Zn, Cu and Fe (Chen et al., 2004a), which may contribute to improved plant growth under some conditions (Garcia-Mina et al., 2004). As an example, Azcona et al. (2011) found superior growth promotion and more rapid maturation of peppers by a compost-derived HS compared with a leonardite-derived HS. Although the compost HS had a higher N-content (7.1%) than the peat-derived HS (1.3%), the authors ruled out nutrient-supply effects (including N) by ensuring adequate chemical fertilisation, and concluded the growth effects were probably a result of the structural organic characteristics of the HS.

It is important to note that the true role of HS in plant signalling is still being strongly debated (Chen et al., 2004a; Trevisan et al., 2010) and the contribution of N-containing residues to plant growth stimulation has not been directly investigated. In addition, HS derived from younger organic material, such as those derived from compost and soils, usually contain a lower molecular weight distribution of molecules/aggregates, which has also been implicated in initiating plant growth responses. Regardless, what is agreed is that there is a need for detailed chemical and spectroscopic characterization of HS to ensure that suitable comparisons can be made between different studies (Canellas et al., 2012; Trevisan et al., 2010). In this respect, it is difficult to make a concrete conclusion on the cause for the rate-response dynamics of plants to soil-derived HS observed here.

3.4 Environmental conditions

Environmentally stressful conditions, such as salinity, heavy metal toxicity or nutrient deficiency, rather than the plant type, played a more prominent role in shaping the root growth response to HS. This finding is especially relevant to the agronomic use of HS, because soil degradation, climate change and diminishing water and nutrient resources are becoming increasingly important constraints to agricultural production, and recommendations for using HS are often directed at alleviating these stresses (Billingham, 2012). Although application rates greater than 100-200 mg $kg^{-1}$of brown coal-derived HS generally inhibited root growth under stressful conditions, the stress condition under which these types of HS were applied was limited to micronutrient deficiency. In comparison, higher application rates of soil-derived HS actually improved root growth; but the stress conditions under which these HS were applied did not include micronutrient deficiency, instead involving salinity or heavy metal toxicity. Both these effects can be accounted for by the high cation exchange capacity of HS. On the one hand, high rates of HS (regardless of source) could easily aggravate micronutrient deficiency by depleting the available pool for plant uptake, as highlighted by reduced Zn uptake in the hydroponic studies of (Vaughan and Macdonald, 1976). Conversely, high rates of HS would alleviate heavy metal or salinity stress by binding excess cations. Taking this into consideration, the type of stress, rather than a stress-by-HS source interaction, would therefore be a more important factor in HS efficacy at high rates.

Unfortunately, our capacity to draw further conclusions is limited by the paucity of studies available that characterise plant growth under stressful conditions when treated with low application rates of HS. There is evidence to suggest that under micronutrient deficient conditions low rates of HS can actually assist in mobilising micronutrients, whilst maintaining a capacity to reduce plant uptake of micronutrients at high or toxic levels (Chen et al., 2004a; Garcia-Mina et al., 2004; Stevenson, 1994). More recent studies also show that low rates (<100 mg kg^{-1}) of compost HS can also reduce the severity of plant stress directly, by stimulating an anti-oxidant stress response in roots that effectively primes the plant to resist other stresses (Garcia et al., 2012). Overall, the actual consequence of the interaction between HS source, application rate and stress conditions is likely to arise from both indirect and direct mechanisms. More research is therefore needed in order to quantify and predict the conditions under which specific mechanisms will dominate.

3.5 Plant type

The effect of HS on shoot biomass was not only dependent on the source and rate of application, but also the plant type. Such an interaction is not altogether surprising and has been previously emphasised (Vaughan and Malcolm, 1985). In our synthesis, not only did HS treatment inhibit the shoot growth of woody perennial plants as compared with herbaceous plant species, it also resulted in significantly lower biomass than non-treated controls. Partitioning of the data set showed that only BC- and peat-derived HS were applied to woody perennials, rather than compost- or soil-derived HS, which may have inflated the difference between plant types. Furthermore, the fact that the number of studies examining woody perennials was low (ns=3) and the rates of BC-derived HS used on this plant type were relatively high (>300 mg kg), means that these results are not fully representative of HS interaction with woody perennials. Nevertheless, in each of these studies a dose-response trend was observed (Kelting et al., 1998; Marino et al., 2008; Vallini et al., 1993), implying a causal relationship and again emphasising the importance of application rate in determining the growth response.

With regards to broad-acre cropping, a more useful distinction would concern differences between monocotyledonous and dicotyledonous plant species. Although our results only suggest marginal differences between monocots and dicots, there is some evidence in the literature showing clear differences between plant types. It is possible that some differences between plant types are related to the inherent susceptibilities to particular soil conditions, especially micronutrient availability. For example, Garcia-Mina et al. (2004) found that the effects of a Zn–HS complex on the shoot and root dry weight of alfalfa under Zn-deficient conditions were significantly positive, but not so in wheat. They speculated that the results reflected the greater sensitivity of alfalfa to Zn deficiency. In contrast, Dobbss et al. (2010) found that the optimum concentration of HS required to stimulate root branching in maize was approximately half that required for maximum stimulation of the dicots tomato and Arabidopsis, suggesting a greater efficacy toward monocots.

4. Practical use of HS in agriculture

4.1 Direct application

A key decision for a farmer or land-holder in applying any soil or plant amendment is the rate at which it should be applied in for maximum efficacy at minimum cost. According to Quilty and Cattle (2011), the cost of HS is in the range of \$40-800 t^{-1}. At application rates of 100 mg kg^{-1}, approximately equivalent to 100 kg ha^{-1} in topsoil (10 cm incorporation), this translates to costs in the range of \$4-80 ha^{-1}. In comparison, the cost of N fertilizer is approximately \$1000 t^{-1} (per unit of N) (USDA, 2012), such that 100 kg N ha^{-1} translates to a cost of approximately \$100 ha^{-1}. Considering that the yield response of crops to N-fertilizer is consistent and profitable (Liu et al., 2006), the use of HS at rates higher than 100 mg kg^{-1} for the sole purpose of short-term increases in biomass productivity in non-compromised soils is unlikely to be competitive with conventional fertilizer practices at current prices. Compost-derived HS, which significantly increase plant growth response at high rates, may be cost-effective if the waste is produced locally and is available at little or no cost. Even so, the additional step of isolating HS from the compost would likely be more of a hindrance than the alternative option of spreading solid compost directly. The results of our study also caution against using high rates of HS on woody perennials because of potential growth inhibition, although more research is needed for this recommendation to be conclusive.

Despite the lack of incentive for applying high rates of HS under satisfactory growth conditions, it may be justified in certain instances where environmental conditions are a constraint to plant growth. Indeed, our synthesis indicates that HS may be most efficacious under stress conditions. Amelioration of saline soils or soils contaminated with heavy metals with HS appears to have positive growth effects on plants, and could assist in reclaiming marginal lands with these characteristics. The effectiveness of HS for assisting plants to tolerate or overcome stress could also extend to conditions of drought (Zhang and Schmidt, 2000) or pathogen control (Loffredo et al., 2008), but these possibilities could not be addressed by the data available in our study. In any case, care should be taken to identify the environmental constraint and ensure that the stress is not exacerbated; for example HS application greater than 100 mg kg^{-1} to micronutrient-deficient soils may actually further inhibit, rather than stimulate, plant growth.

The question then remains; are low rates (<100 mg kg^{-1}) of HS application efficacious and if so, economically and practically worthwhile? To answer this question we focussed on HS derived from brown coals, as these usually form the basis of commercial products. We found that the growth response to low rates of brown coal-derived HS is non-linear and is more appropriately described by the sum of two quadratic functions rather than a single quadratic or higher polynomial (Figure 8). An initial sharp peak in growth response is observed between 5-40 mg kg^{-1} with a maximum around 20 mg kg^{-1}, followed by a more gradual growth increase from 40-200 mg kg^{-1}. There appears to be a greater opportunity to maximise plant growth promotion by applying brown coal-derived HS in the lower range (5-40 mg kg^{-1}) of the initial peak, which would also be more economically rational. Based on the extrapolation of a number of studies, Chen et al. (2004b) calculated the amount of HS required for an effective soil application to be 22.5 mg kg^{-1}, equivalent to 75 mg L^{-1} of HS dispersed in a soil at a moisture content of 30%. This value is very close to the peak of the low-range quadratic response calculated here.

Unfortunately, the reasons for growth promotion at these low rates cannot be directly determined through meta-analysis of the data collated here. As outlined earlier, the interaction of HS with plant essential elements, including N, P and micronutrients, is known to improve nutrient availability and may be one reason for growth promotion at low rates. If this is correct, a substantial agronomic opportunity therefore exists to improve the efficiency of fertilizer nutrient use, rather than enhancing growth *per se*. There is also evidence that HS can positively interact with beneficial microorganisms, offering the possibility of additional productivity gains if harnessed appropriately.

4.2 Application as synergists

The chemical properties of HS, including hydrophilic and hydrophobic domains and zwitterionic features, facilitate interactions with a wide variety of soil constituents. Theoretically, these properties act to buffer biological susceptibility to nutritional extremes, such that high activities of salts, metals and protons in the soil solution can be reduced, whilst low activities of nutrients are mobilised into plant-available forms. Recent research has demonstrated the potential for exploiting these properties of HS to design slow- or controlled-release fertilizers that better match the availability of nutrients to the plant lifecycle (Davidson and Gu, 2012).

Nitrogen fertilizers coated with humic acids are commercially available and are reported to increase fertilizer use efficiency (Chen et al., 2008), probably through a number of mechanisms. First, HS have been shown to significantly reduce urea hydrolysis from urea-ammonium nitrate (UAN) and also retard the formation of NO_3^-, implying urease- and nitrification-inhibition activity (Alkanani et al., 1990). Reduced urea hydrolysis in HS-treated soils has been linked to biological buffering of the HS on microbial populations and enzyme activities (Dong et al., 2009). However, GarciaSerna et al. (1996) also showed that humic acids (1% w/w) sprayed onto the surface of urea or nitrophoska granules slow nitrogen release through physico-chemical mechanisms, but probably not solely by acting as a physical coating since the release curve was observed to be convex, not concave. Aside from slowing the formation and release of ammonium (NH_4^+) from urea, HS can also reduce the volatilization of ammonia without significantly altering pH, which is one of the main drivers of NH_3 emission (Kasim et al., 2009). Erro et al. (2007) formulated a compound HS-NPK fertiliser and observed reduced ammonia volatilisation, reduced N-leaching, and increased plant growth with respect to an NPK control fertiliser. However, Kiran et al. (2010) found that humic-coated urea did not improve N-use efficiency in rice paddy systems, whereas a number of other controlled-release fertilizer formulations were effective.

There is also evidence that the association of soluble phosphate with HS reduces its binding and precipitation in soil, allowing for greater plant uptake (Alvarez et al., 2004; Hua et al., 2008; Schefe et al., 2008). Indeed Gerke (2010) suggests that the majority of bicarbonate-extractable P (e.g. Olsen-P, Colwell-P) actually exists in soil as humic-metal-P, rather than free or sorbed orthophosphate, but becomes liberated by acidification steps during analysis. On the basis of these reports, Erro and co-workers (Erro et al., 2012; Erro et al., 2009) developed and characterised the performance of several 'organic complexed superphosphate (CSP)' fertilisers. The CSPs were produced by introducing HS during the chemical synthesis of single-super phosphate (SSP). Glasshouse experiments showed that CSPs consistently enhanced P-accumulation in wheat grown in both acid and alkaline P-fixing soils when compared against a SSP control treatment. The authors suggested that greater P-uptake efficiency afforded by the CSPs is related to the formation of stable monocalcium – phosphate-humic complexes during CSP preparation.

Together, these results suggest a role for the use of HS in improving N- and P-use efficiency in cropping systems, but more work is needed in developing effective formulations. The potential for using HS to improve micronutrient availability and absorption is also well recognized (Chen et al., 2004a; Garcia-Mina et al., 2004), but there is a noticeable lack of experimental studies reporting the efficacy of micronutrient-HS fertilizer formulations. In fact, one recent study showed that HS-Fe complexes were ineffective at supplying iron to Fe-deficient soybean, either as a foliar spray or through root absorption, whereas synthetic chelates (e.g. EDTA) were effective at delivering Fe (Rodriguez-Lucena et al., 2010). Because there are an ever increasing number of humic-coated or humic-containing fertilizers on the market, further research and validation of such products is urgently needed to provide farmers with reliable information for making agronomic decisions.

Another strategy gaining popularity in sustainable agronomy is the use of microbial inoculants in agriculture as plant-growth promoters (PGPs). These PGPs can assist in nutrient acquisition, stress tolerance and pathogen suppression through diverse biological functions. Although substantial work has been done in this area, little is known about the interactions of PGPs with indigenous or exogenous HS. To our knowledge, only one study has directly examined the potential to use HS in conjunction with PGPR for stimulating plant growth (Canellas et al., In Press). These authors speculated that the auxin-like action of HS could improve the colonization ability of PGPR via root-branching nodes, as has been previously observed with the synthetic auxin 2,4-D (Katupitiya et al., 1995). They found that co-inoculation of *Herbaspirillum seropedicae* with 20 mg HS C L^{-1} improved colonization of maize roots, but that this effect was dose dependent and that colonization was inhibited at a higher HS concentration. Validation of this effect in the field confirmed a synergistic effect, with maize yield increasing an additional 45-48% when HS and *H. seropedicae* were applied in combination, compared with sole treatments of *H. seropedicae* or HS, respectively. The authors caution against generalising this result until further studies can be performed, but their results clearly warrant more research in this area with other PGPR strains.

It would also be interesting to extend this research to the effects of HS on plant-microbial symbioses, such as rhizobial and mycorrhizal associations. These co-operative plant microbial associations are critical components of nutrient cycling in agro-ecosystems and enhance plant nutrient acquisition (Peoples and Craswell, 1992; Smith and Read, 2008; Zhu et al., 2001). Although Vallini et al. (1993) reported a depression in the mycorrhizal colonization of laurel roots and hyphal length in the presence of high concentrations of HS (>800 mg kg^{-1}), a more recent study by Gryndler et al. (2005) found that HS applied in hydroponics at a rate of approximately 800 mg L^{-1} stimulated maize root colonization and production of extraradical mycelium by the mycorrhizal fungus *Glomus claroideum* BEG 23. In a similar fashion, Gaur and Bhardwaj observed a greater nodule formation in the legume *Sesbania aculeata* by native rhizobia when sodium humate was amended into soil at a rate of 600 mg ka^{-1}. Unfortunately, as Gryndler et al. (2005) acknowledged, experiments on the effects of HS on plant-microbial symbioses are rare and it is difficult to make any consistent conclusions.

5. Knowledge gaps and research needs

Through the process of meta-analysis, a number of knowledge gaps have also been identified. First, the majority of papers reporting experiments on HS lack information about the organic structure, molecular nature and size, and mineral concentrations of the HS amendments. Considering the importance of HS source shown by our analysis, provision of this kind of information in future studies will be necessary in order to increase our understanding of how particular HS improve plant growth. Such knowledge may subsequently open the door to tailoring HS products for specific purpose. In conjunction with HS characteristics, more complete meta-data about the environmental conditions under which the HS are applied are needed. Data about the soil, such as the nature of native organic matter, pH, EC, texture and mineral nutrient concentrations are required to increase our understanding of HS-soil-plant interactions. The focus of most studies conducted in soil is on the HS applied and few if any have attempted to reconcile the possible interactions between HS already in the soil (where HS levels would be much higher) and those applied. The complexity of scientifically addressing this research question is possibly why data is lacking. In addition, there is the recognised fact that the extraction processes, generally involving alkaline treatments, are likely to have chemically modified the original organic materials (Swift et al., 1996).

In terms of agronomic management, HS application rate is a critical decision that can not only affect plant growth, but also economic margins. More work is needed involving valid, scientifically designed field trials with crops grown to harvest, in order to define application rate windows that will maximise growth whilst minimising the risk of economic loss. Interestingly, the majority of studies reviewed here only measured growth responses for less than 3 months during early vegetative growth. It remains unknown if the trends observed in the early stages of plant growth will be maintained for the duration of the plant life cycle and therefore translates into yield gains at harvest. This knowledge gap is currently being further pursued by the authors.

The use of HS in conjunction with inorganic fertilisers is of direct relevance here, as HS may improve nutrient recovery by plants without enhancing growth *per se*, leading to reduced fertiliser input costs. There is also a real need to systematically determine the effects of adding HS on soil microbes and related carbon and nutrient cycling. Much of the work focussed on the effect of HS on nutrient acquisition by plants has been conducted in hydroponic systems and may therefore overlook the importance on plant-microbial associations in the rhizosphere.

Finally, there is a paucity of data surrounding the long-term effects of HS, or of repeated HS application. The majority of studies reported here had durations of less than 6 months, and in many cases were only observed over daily or weekly timeframes. Any improvements to soil quality are likely to occur over longer periods and effects on crop growth may not be quantifiable in the short term, in accordance with long-term studies of other ‘organic’ agronomy practices (Clark et al., 1998; Gosling and Shepherd, 2005).

6. Conclusions

This meta-analysis has shown that the growth response of plants to HS, although generally positive, is influenced by a number of environmental and management factors. Our findings indicate that the source of the HS in particular will have a strong impact on whether or not plant growth is significantly improved. Plant type and stress conditions also influence the plant growth response to HS, but to a lesser extent. Interactions between each of these factors and the HS application rate also moderate the plant growth response, emphasizing the complexity of obtaining predictable responses. More research is needed to characterise the structure-activity relations of HS, and how these can be exploited either through direct application or application as synergists with chemical or biological fertilisers. We conclude by reiterating that the prospects for using HS as plant growth stimulants in agricultural systems are theoretically strong, but continued research and extension is needed to realise their full potential under diverse environmental conditions.

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Table 1. Significance (p-value) of models containing a single moderator, according to plant shoot or root dry weight response to HS application. Significant models are shown in bold ($p<0.05$).

Moderator	Shoot Growth	Root Growth
None [a]	**<0.001**	**<0.001**
Media	0.336	**0.016**
Stress	**0.015**	0.144
Plant type	**0.026**	**0.031**
Application location	0.380	0.063
Application rate	**0.002**	0.261
Source	**<0.001**	**<0.001**

[a] Overall random-effect model without any moderators; significance indicates statistical difference between HS-treated plants and control (no HS treatment) plants.

Table 2. Pairwise interactions between explanatory variables in the optimised BRT model. Higher numbers indicate increased importance of the interaction for predicting a growth response. Grey cells are interactions contributing to RDW response; white cells are interactions contributing to SDW response. Numbers in bold show the two most important interactions.

	Media	Stress	Plant	HS Source	Application Site	Application Rate
Media		2.38	0.03	0.82	0.03	2.72
Stress	0.39		1.23	**7.89**	0.86	1.73
Plant	0.05	2.93		0.94	0.27	0.65
HS Source	0.41	0.3	0.96		0.57	**5.38**
Application Site	0.37	0.24	0.18	0.63		0.76
Application Rate	0.11	0.24	**4.93**	**7.18**	0.77	

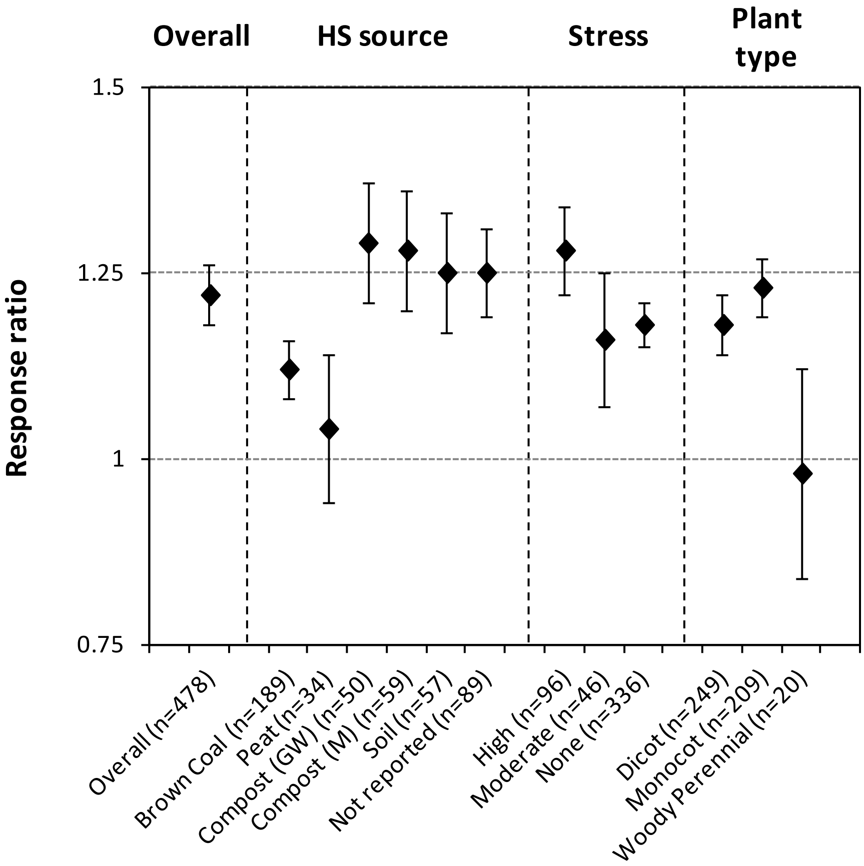


Figure 1. Estimated shoot growth response (weighted mean ± 95% confidence level) of plants to HS application for three significant explanatory moderators. Ratios > 1 indicate growth promotion; < 1 indicate growth suppression. The number of data points in each group is given in parentheses.

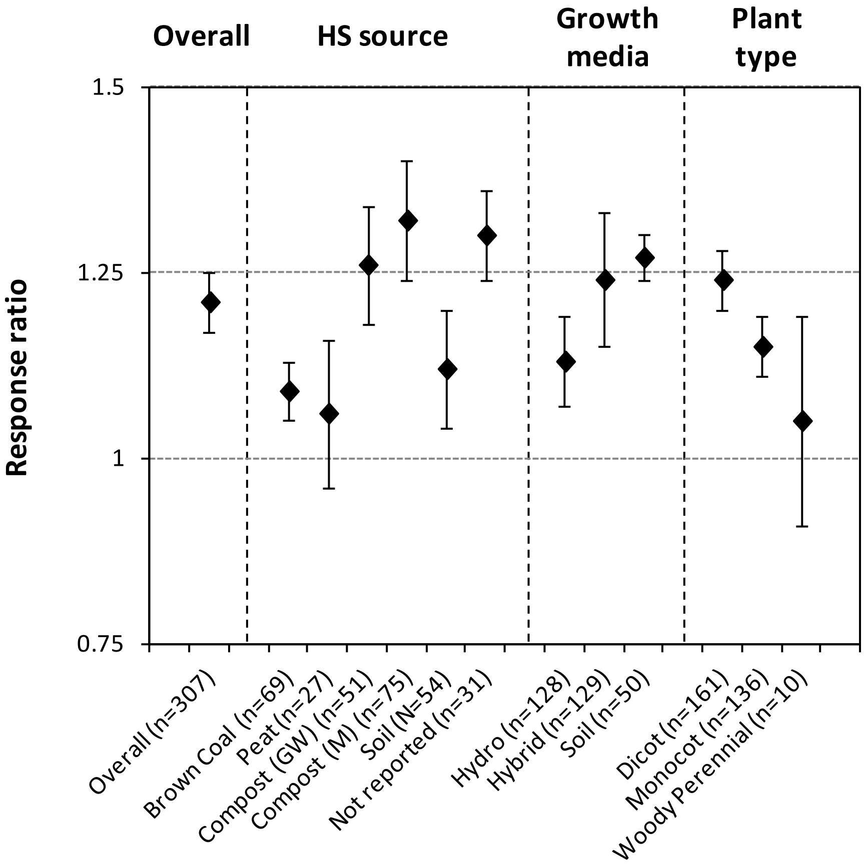
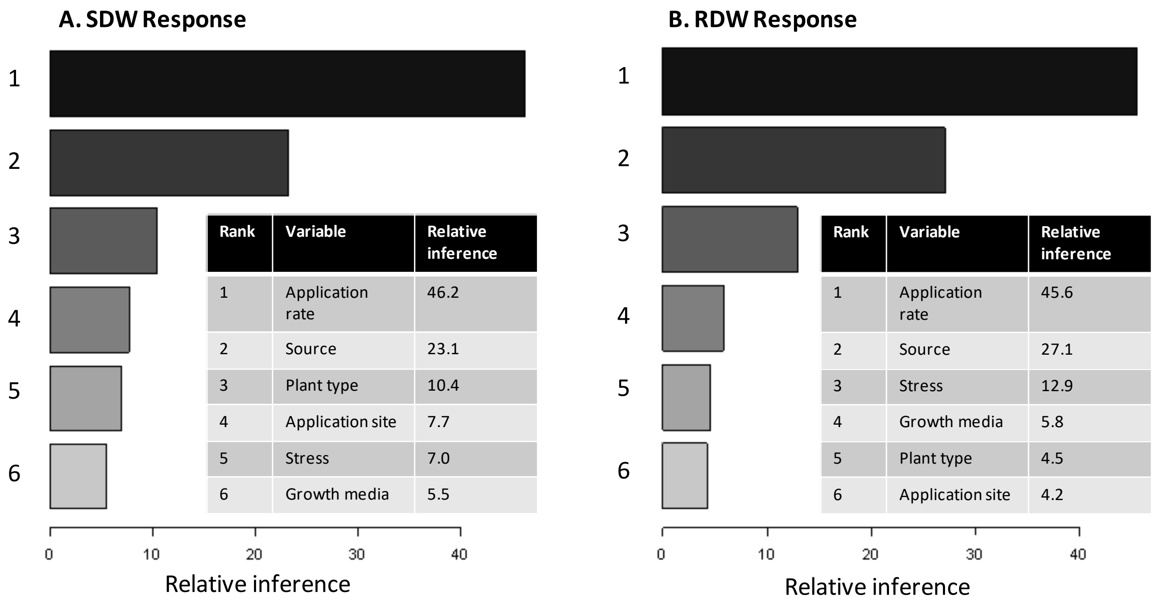


Figure 2. Estimated shoot growth response (weighted mean ± 95% confidence level) of plants to HS application for three significant explanatory moderators. Ratios > 1 indicate growth promotion; < 1 indicate growth suppression. The number of data points in each group is given in parentheses.



A. SDW Response

Rank	Variable	Relative inference
1	Application rate	46.2
2	Source	23.1
3	Plant type	10.4
4	Application site	7.7
5	Stress	7.0
6	Growth media	5.5

B. RDW Response

Rank	Variable	Relative inference
1	Application rate	45.6
2	Source	27.1
3	Stress	12.9
4	Growth media	5.8
5	Plant type	4.5
6	Application site	4.2

Figure 3. Relative contribution of explanatory variables to the optimum boosted regression tree model.

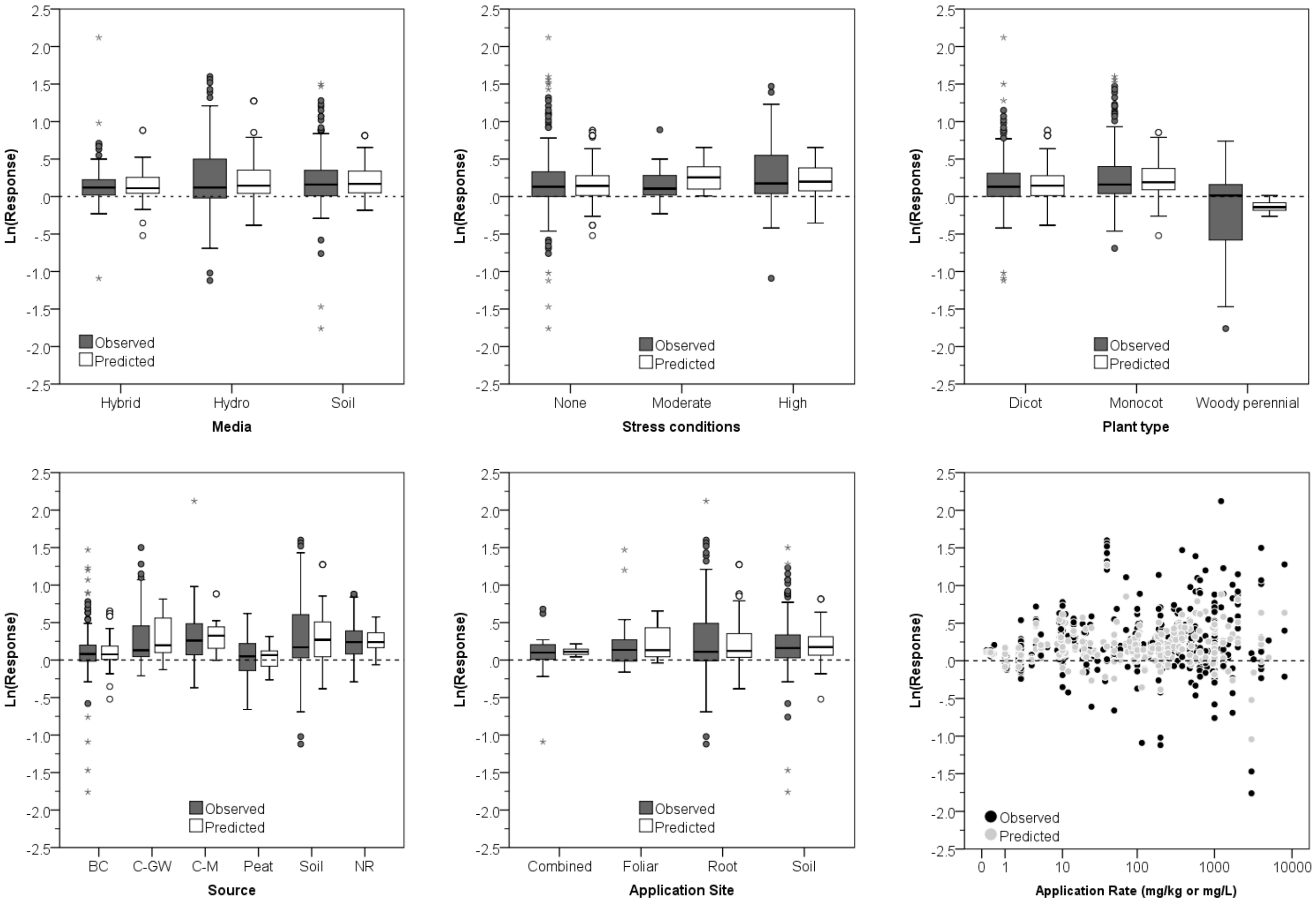


Figure 4. Modelled distribution of SDW response, grouped by explanatory predictor. Boxplots show median values (solid bold horizontal lines), 25th-75th quartiles (box), 1.5 times the interquartile range (whiskers), outliers (circle points) and extreme outliers (star points). Abbreviations for the HS source are brown coal (BC); green waste compost (CGW), manure compost (CM) and not reported (NR).

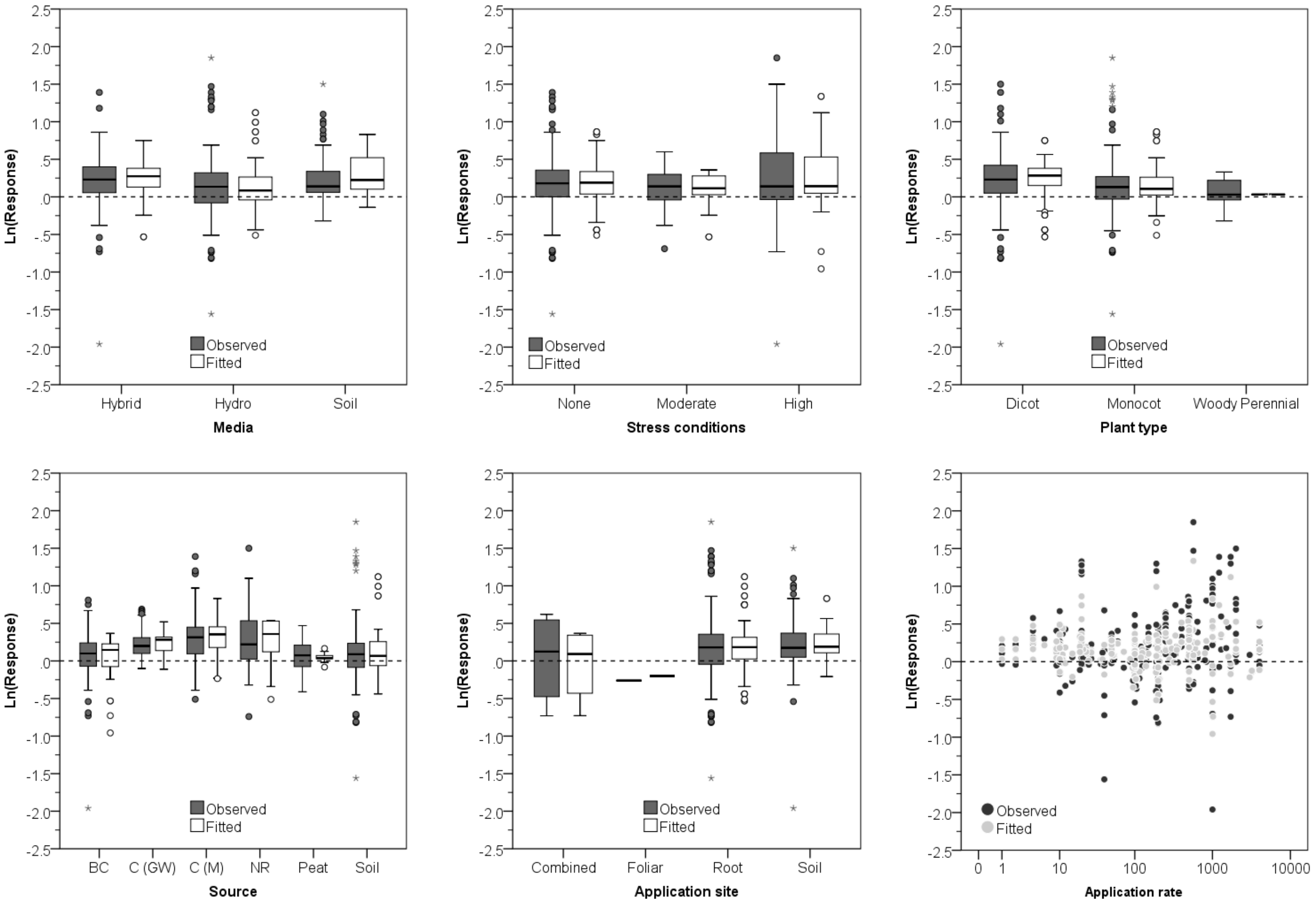


Figure 5. Modelled distribution of RDW response, grouped by explanatory predictor. Boxplots show median values (solid bold horizontal lines), 25th-75th quartiles (box), 1.5 times the interquartile range (whiskers), outliers (circle points) and extreme outliers (star points). Abbreviations for the HS source are brown coal (BC); green waste compost (CGW), manure compost (CM) and not reported (NR).

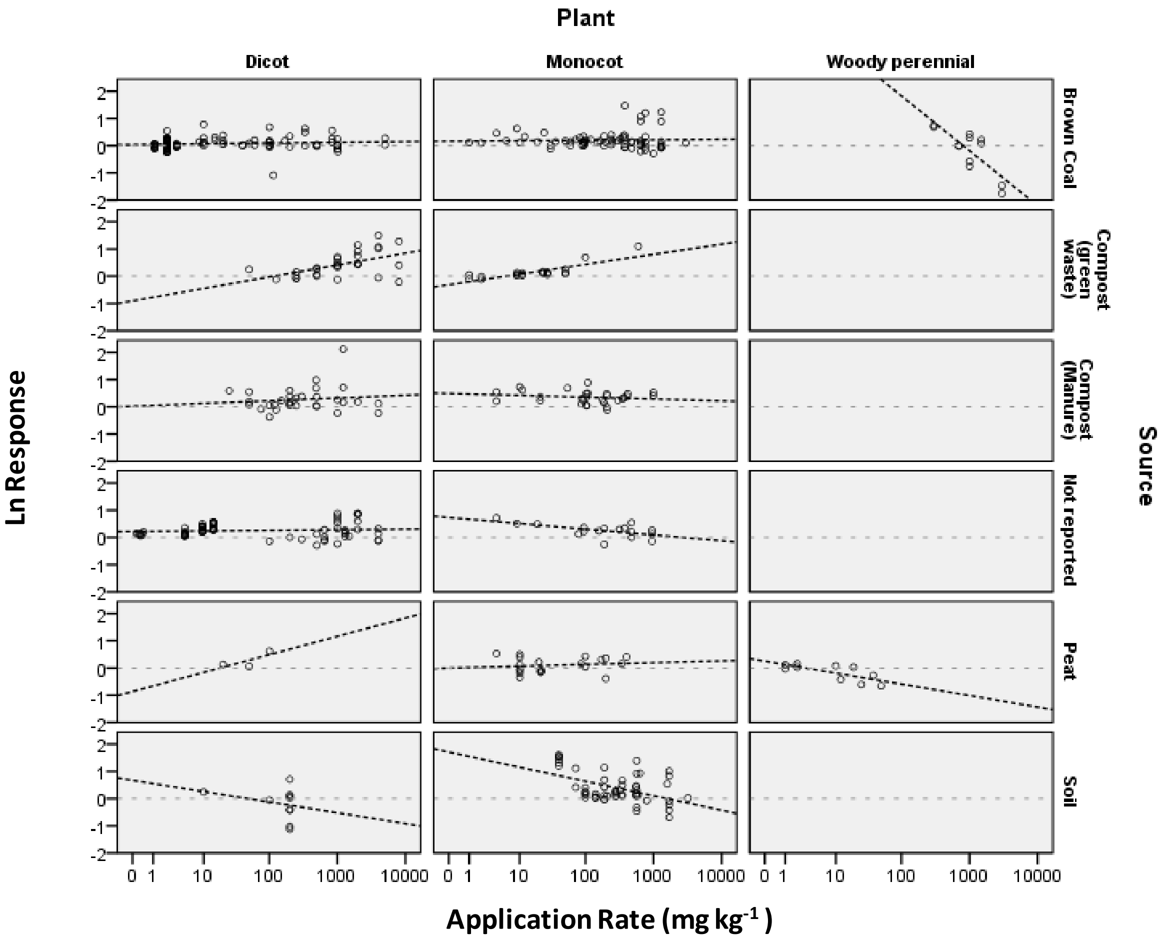


Figure 6. Effect of application rate on SDW response under different scenarios of HS source and plant type. The black dashed lines show linear fits to the data and have been superimposed to aid interpretation. Data above the grey dashed line indicate shoot growth promotion by HS; data below indicate shoot growth suppression.

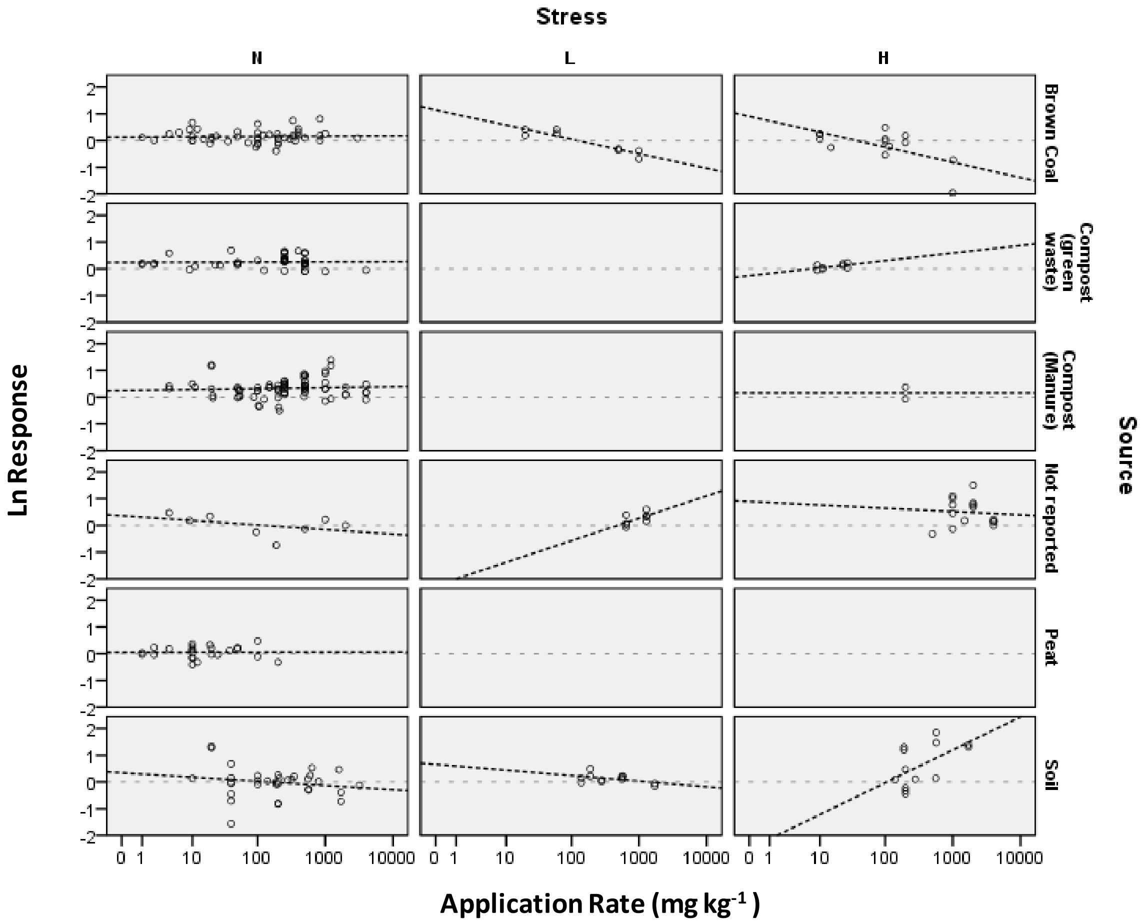


Figure 7. Effect of application rate on RDW response under different scenarios of HS source and plant type. Stress levels are given as: no stress (N), moderate stress (L) and high stress (H). The black dashed lines show linear fits to the data and have been superimposed to aid interpretation. Data above the grey dashed line indicate shoot growth promotion by HS; data below indicate shoot growth suppression.

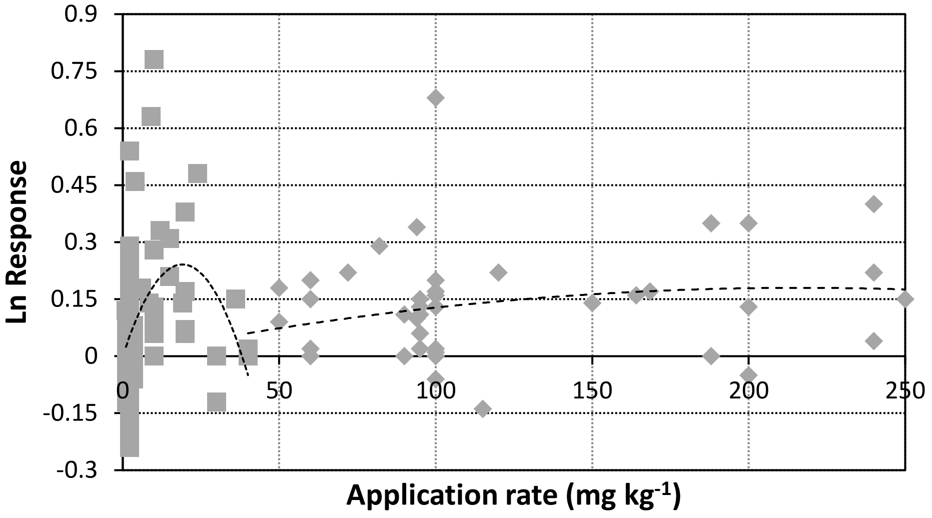


Figure 8. Effect of application rate of brown coal-derived HS on SDW. Dashed lines show quadratic fits to rates less than 50 mg kg^{-1} (short dash) and rates greater than or equal to 50 mg kg^{-1} (long-dash).

Appendix 1. Study references for SDW data used in the meta-analysis.

No.	ID	Authors	Title	Year	Source title	Vol.	Iss.	Page start	Page end
1	5	Azcona, I., Pascual, I., Aguirreolea, J., Fuentes, M., Garcia-Mina, J., Sanchez-Diaz, M.	Growth and development of pepper are affected by humic substances derived from composted sludge	2011	Journal of Plant Nutrition and Soil Science	174	6	916	924
2	6	Ertani, A., Francioso, O., Tugnoli, V., Righi, V., Nardi, S.	Effect of commercial lignosulfonate-humate on Zea mays L. metabolism	2011	Journal of Agricultural and Food Chemistry	59	22	11940	11948
3	14	Yarkova, T.A.	Chemical modification of humic acids by the introduction of indole-containing fragments	2011	Solid Fuel Chemistry	45	4	261	266
4	25	Saruhan, V., Kusvuran, A., Kokten, K.	The effect of different replications of humic acid fertilization on yield performances of common vetch (Vicia sativa L.)	2011	African Journal of Biotechnology	10	29	5587	5592
5	38	Tahir, M.M., Khurshid, M., Khan, M.Z., Abbasi, M.K., Kazmi, M.H.	Lignite-derived humic acid effect on growth of wheat plants in different soils	2011	Pedosphere	21	1	124	131
6	38	Tahir, M.M., Khurshid, M., Khan, M.Z., Abbasi, M.K., Kazmi, M.H.	Lignite-derived humic acid effect on growth of wheat plants in different soils	2011	Pedosphere	21	1	124	131
7	15	Obsuwan, K., Namchote, S., Sanmanee, N., Panishkan, K., Dharmvanij, S.	Effect of various concentrations of humic acid on growth and development of eggplant seedlings in tissue cultures at low nutrient level	2011	Proceedings of World Academy of Science, Engineering and Technology	80		276	278
8	32	Khaled, H., Fawy, H.	Effect of different Levels of humic acids on the nutrient content, plant growth, and soil properties under conditions of salinity	2011	Soil and Water Research	6	1	21	29
9	41	Cordeiro, F.C., Santa-Catarina, C., Silveira, V., De Souza, S.R.	Humic acid effect on catalase activity and the generation of reactive oxygen species in corn (Zea mays)	2011	Bioscience, Biotechnology and Biochemistry	75	1	70	74
10	42	Morard, P., Eyheraguibel, B., Morard, M., Silvestre, J.	Direct effects of Humic-Like substance on growth, water, and mineral nutrition of various species	2011	Journal of Plant Nutrition	34	1	46	59

11	44	Celik, H., Katkat, A.V., Asik, B.B., Turan, M.A.	Effect of foliar-applied humic acid to dry weight and mineral nutrient uptake of maize under calcareous soil conditions	2011	Communications in Soil Science and Plant Analysis	42	1	29	38
12	61	Cimrin, K.M., Turkmen, O., Turan, M., Tuncer, B.	Phosphorus and humic acid application alleviate salinity stress of pepper seedling	2010	African Journal of Biotechnology	9	36	5845	5851
13	62	Baldotto, L.E.B., Baldotto, M.A., Canellas, L.P., Bressan-Smith, R., Olivares, F.L.	Growth promotion of pineapple 'Vitoria' by humic acids and burkholderia spp. during acclimatization	2010	Revista Brasileira de Ciencia do Solo	34	5	1593	1600
14	63	Gulser, F., Sonmez, F., Boysan, S.	Effects of calcium nitrate and humic acid on pepper seedling growth under saline condition	2010	Journal of Environmental Biology	31	5 SUPPL.	873	876
15	67	Paksoy, M., Turkmen, O., Dursun, A.	Effects of potassium and humic acid on emergence, growth and nutrient contents of okra (Abelmoschus esculentus L.) seedling under saline soil conditions	2010	African Journal of Biotechnology	9	33	5343	5346
16	70	Petrus, A.C., Ahmed, O.H., Muhamad, A.M.N., Nasir, H.M., Jiwan, M.	Effect of K-N-humates on dry matter production and nutrient use efficiency of maize in Sarawak, Malaysia	2010	TheScientificWorldJournal	10		1282	1292
17	58	Asli, S., Neumann, P.M.	Rhizosphere humic acid interacts with root cell walls to reduce hydraulic conductivity and plant development	2010	Plant and Soil	336	1	313	322
18	78	Hartz, T.K., Bottoms, T.G.	Humic substances generally ineffective in improving vegetable crop nutrient uptake or productivity	2010	HortScience	45	6	906	910
19	80	Mora, V., Bacaicoa, E., Zamarreno, A.-M., Aguirre, E., Garnica, M., Fuentes, M., Garcia-Mina, J.-M.	Action of humic acid on promotion of cucumber shoot growth involves nitrate-related changes associated with the root-to-shoot distribution of cytokinins, polyamines and mineral nutrients	2010	Journal of Plant Physiology	167	8	633	642
20	51	Kirn, A., Kashif, S.R., Yaseen, M.	Using indigenous humic acid from lignite to increase growth and yield of okra (Abelmoschus esculentus L.)	2010	Soil and Environment	29	2	187	191
21	52	El-Hefny, E.M.	Effect of saline irrigation water and humic acid application on growth and productivity of two cultivars of cowpea (Vigna unguiculata L. Walp)	2010	Journal of Applied Sciences Research	6	12	6154	6168
22	99	Bandiera, M., Mosca, G., Vamerali, T.	Humic acids affect root characteristics of fodder radish (Raphanus sativus L. var. oleiformis Pers.) in metal-polluted wastes	2009	Desalination	246	1-3	78	91

23	97	Asik, B.B., Turan, M.A., Celik, H., Katkat, A.V.	Effects of humic substances on plant growth and mineral nutrients uptake of wheat (Triticum durum cv. Salihli) under conditions of salinity	2009	Asian Journal of Crop Science	1	2	87	95
24	100	Kasim, S., Ahmed, O.H., Majid, N.M.A., Yusop, M.K., Jalloh, M.B.	Effect of organic based N fertilizer on dry matter (Zea mays L.), Ammonium and nitrate recovery in an acid soil of Sarawak, Malaysia	2009	American Journal of Applied Sciences	6	7	1289	1294
25	112	Nikbakht, A., Kafi, M., Babalar, M., Xia, Y.P., Luo, A., Etemadi, N.-A.	Effect of humic acid on plant growth, nutrient uptake, and postharvest life of gerbera	2008	Journal of Plant Nutrition	31	12	2155	2167
26	126	Eyheraguibel, B., Silvestre, J., Morard, P.	Effects of humic substances derived from organic waste enhancement on the growth and mineral nutrition of maize	2008	Bioresource Technology	99	10	4206	4212
27	127	De Santiago, A., Quintero, J.M., Carmona, E., Delgado, A.	Humic substances increase the effectiveness of iron sulfate and Vivianite preventing iron chlorosis in white lupin	2008	Biology and Fertility of Soils	44	6	875	883
28	132	Clapp, C.E., Shenker, M., Hayes, M.H.B., Liu, R., Cline, V.W., Palazzo, A.J., Chen, Y.	Microsystems for rapid evaluation of plant growth response to organic amendments	2008	Soil Science	173	5	342	349
29	141	Willis, J.M., Hester, M.W.	Evaluation of enhanced Panicum amarum establishment through fragment plantings and humic acid amendment	2008	Journal of Coastal Research	24	2 SUPPL. B	263	268
30	128	Marino, G., Francioso, O., Carletti, P., Nardi, S., Gessa, C.	Mineral content and root respiration of in vitro grown kiwifruit plantlets treated with two humic fractions	2008	Journal of Plant Nutrition	31	6	1074	1090
31	116	Celik, H., Katkat, A.V., Asik, B.B., Turan, M.A.	Effects of soil applied humic substances to dry weight and mineral nutrients uptake of maize under calcareous soil conditions	2008	Archives of Agronomy and Soil Science	54	6	605	614
31	123	Canellas, L.P., Zandonadi, D.B., Busato, J.G., Baldotto, M.A., Simoes, M.L., Martin-Neto, L., Facanha, A.R., Spaccini, R., Piccolo, A.	Bioactivity and chemical characteristics of humic acids from tropical soils sequence	2008	Soil Science	173	9	624	637
32	174	Khan, S., Cao, Q., Chen, B.-D., Zhu, Y.-G.	Humic acids increase the phytoavailability of Cd and Pb to wheat plants cultivated in freshly spiked, contaminated soil	2006	Journal of Soils and Sediments	6	4	236	242

33	196	Gryndler, M., Hrselova, H., Sudova, R., Gryndlerova¡, H., Rezacova, V., Merhautova, V.	Hyphal growth and mycorrhiza formation by the arbuscular mycorrhizal fungus Glomus claroideum BEG 23 is stimulated by humic substances	2005	Mycorrhiza	15	7	483	488
34	216	Turkmen, O., Dursun, A., Turan, M., Erdinc, C.	Calcium and humic acid affect seed germination, growth, and nutrient content of tomato (Lycopersicon esculentum L.) seedlings under saline soil conditions	2004	Acta Agriculturae Scandinavica Section B: Soil and Plant Science	54	3	168	174
36	242	Sharif, M., Khattak, R.A., Sarir, M.S.	Effect of different levels of lignitic coal derived humic acid on growth of maize plants	2002	Communications in Soil Science and Plant Analysis	33	19-20	3567	3580
37	245	Badora, A.	Bioaccumulation of Al, Mn, Zn and Cd in Pea Plants (Pisum sativum L.) Against a Background of Unconventional Binding Agents	2002	Polish Journal of Environmental Studies	11	2	109	116
38	252	Atiyeh, R.M., Lee, S., Edwards, C.A., Arancon, N.Q., Metzger, J.D.	The influence of humic acids derived from earthworm-processed organic wastes on plant growth	2002	Bioresource Technology	84	1	7	14
39	253	Masciandaro, G., Ceccanti, B., Ronchi, V., Benedicto, S., Howard, L.	Humic substances to reduce salt effect on plant germination and growth	2002	Communications in Soil Science and Plant Analysis	33	3-4	365	378
40	276	Bidegain, R.A., Kaemmerer, M., Guiresse, M., Hafidi, M., Rey, F., Morard, P., Revel, J.C.	Effects of humic substances from composted or chemically decomposed poplar sawdust on mineral nutrition of ryegrass	2000	Journal of Agricultural Science	134	3	259	267
41	300	Adani, F., Genevini, P., Zaccheo, P., Zocchi, G.	The effect of commercial humic acid on tomato plant growth and mineral nutrition	1998	Journal of Plant Nutrition	21	3	561	575
42	298	Kelting, M., Harris, J.R., Fanelli, J., Appleton, B.	Biostimulants and soil amendments affect two-year posttransplant growth of red maple and Washington hawthorn	1998	HortScience	33	5	819	822
43	307	Loffredo, E., Senesi, N., D'Orazio, V.	Effects of humic acids and herbicides, and their combinations on the growth of tomato seedlings in hydroponics	1997	Journal of Plant Nutrition and Soil Science	160	5	455	461
44	305	Ayuso, M., Moreno, J.L., Hernandez, T., Garcia, C.	Characterisation and evaluation of humic acids extracted from urban waste as liquid fertilisers	1997	Journal of the Science of Food and Agriculture	75	4	481	488
45	325	Ayuso, M., Hernandez, T., Garcia, C., Pascual, J.A.	Stimulation of barley growth and nutrient absorption by humic substances originating from various organic materials	1996	Bioresource Technology	57	3	251	257
46	322	Gonet, S.S., Dziamski, A., Gonet, E.	Application of humus preparations from oxyhumolite in crop production	1996	Environment International	22	5	559	562

47	315	Ayuso, M., Hernandez, T., Garcia, C., Pascual, J.A.	A comparative study of the effect on barley growth of humic substances extracted from municipal wastes and from traditional organic materials	1996	Journal of the Science of Food and Agriculture	72	4	493	500
48	327	Valdrighi, M.M., Pera, A., Agnolucci, M., Frassinetti, S., Lunardi, D., Vallini, G.	Effects of compost-derived humic acids on vegetable biomass production and microbial growth within a plant (Cichorium intybus)-soil system: A comparative study	1996	Agriculture, Ecosystems and Environment	58	2-3	133	144
49	343	Vallini, G., Pera, A., Avio, L., Valdrighi, M., Giovannetti, M.	Influence of humic acids on laurel growth, associated rhizospheric microorganisms, and mycorrhizal fungi	1993	Biology and Fertility of Soils	16	1	1	4
50	341	Piccolo, A., Celano, G., Pietramellara, G.	Effects of fractions of coal-derived humic substances on seed germination and growth of seedlings (Lactuga sativa and Lycopersicum esculentum)	1993	Biology and Fertility of Soils	16	1	11	15
51	346	Lobartini, J.C., Tan, K.H., Rema, J.A., Gingle, A.R., Pape, C., Himmelsbach, D.S.	The geochemical nature and agricultural importance of commercial humic matter	1992	Science of the Total Environment	113	1-2	1	15
52	351	Senesi, N., Loffredo, E., Padovano, G.	Effects of humic acid-herbicide interactions on the growth of Pisum sativum in nutrient solution	1990	Plant and Soil	127	1	41	47
53	360	Cabrera, D., Young, S.D., Rowell, D.L.	The toxicity of cadmium to barley plants as affected by complex formation with humic acid	1988	Plant and Soil	105	2	195	204
54	365	Tan, K.H., Binger, A.	Effect of humic acid on aluminum toxicity in corn plants	1986	Soil Science	141	1	20	25
55	376	Tan, K.H., Nopamornbodi, V.	Effect of different levels of humic acids on nutrient content and growth of corn (Zea mays L.)	1979	Plant and Soil	51	2	283	287
56	379	Vaughan, D., Linehan, D.J.	The growth of wheat plants in humic acid solutions under axenic conditions	1976	Plant and Soil	44	2	445	449
57	385	Gaur, A.C., Bhardwaj, K.K.R.	Influence of sodium humate on the crop plants inoculated with bacteria of agricultural importance	1971	Plant and Soil	35	1	613	621

Appendix 2. Study references for RDW data used in the meta-analysis.

No.	ID	Authors	Title	Year	Source title	Vol.	Iss.	Page start	Page end
1	1	Mora, V., Baigorri, R., Bacaicoa, E., Zamarreno, A.M., Garcia-Mina, J.M.	The humic acid-induced changes in the root concentration of nitric oxide, IAA and ethylene do not explain the changes in root architecture caused by humic acid in cucumber	2012	Environmental and Experimental Botany	76		24	32
2	5	Azcona, I., Pascual, I., Aguirreolea, J., Fuentes, M., Garcia-Mina, J., Sanchez-Diaz, M.	Growth and development of pepper are affected by humic substances derived from composted sludge	2011	Journal of Plant Nutrition and Soil Science	174	6	916	924
3	6	Ertani, A., Francioso, O., Tugnoli, V., Righi, V., Nardi, S.	Effect of commercial lignosulfonate-humate on Zea mays L. metabolism	2011	Journal of Agricultural and Food Chemistry	59	22	11940	11948
4	9	Jindo, K., Martim, S.A., Navarro, E.C., Perez-Alfocea, F., Hernandez, T., Garcia, C., Aguiar, N.O., Canellas, L.P.	Root growth promotion by humic acids from composted and non-composted urban organic wastes	2011	Plant and Soil			1	12
5	14	Yarkova, T.A.	Chemical modification of humic acids by the introduction of indole-containing fragments	2011	Solid Fuel Chemistry	45	4	261	266
6	51	Kirn, A., Kashif, S.R., Yaseen, M.	Using indigenous humic acid from lignite to increase growth and yield of okra (Abelmoschus esculentus L.)	2010	Soil and Environment	29	2	187	191
7	61	Cimrin, K.M., Turkmen, O., Turan, M., Tuncer, B.	Phosphorus and humic acid application alleviate salinity stress of pepper seedling	2010	African Journal of Biotechnology	9	36	5845	5851
8	62	Baldotto, L.E.B., Baldotto, M.A., Canellas, L.P., Bressan-Smith, R., Olivares, F.L.	Growth promotion of pineapple 'Vitoria' by humic acids and burkholderia spp. during acclimatization	2010	Revista Brasileira de Ciencia do Solo	34	5	1593	1600
9	63	Gulser, F., Sonmez, F., Boysan, S.	Effects of calcium nitrate and humic acid on pepper seedling growth under saline condition	2010	Journal of Environmental Biology	31	5 SUPPL.	873	876
10	67	Paksoy, M., Turkmen, O., Dursun, A.	Effects of potassium and humic acid on emergence, growth and nutrient contents of okra	2010	African Journal of Biotechnology	9	33	5343	5346

			(Abelmoschus esculentus L.) seedling under saline soil conditions						
11	70	Petrus, A.C., Ahmed, O.H., Muhamad, A.M.N., Nasir, H.M., Jiwan, M.	Effect of K-N-humates on dry matter production and nutrient use efficiency of maize in Sarawak, Malaysia	2010	TheScientificWorldJournal	10		1282	1292
12	84	Busato, J.G., Zandonadi, D.B., Dobbss, L.B., Facanha, A.R., Canellas, L.P.	Humic substances isolated from residues of sugar cane industry as root growth promoter	2010	Scientia Agricola	67	2	206	212
13	93	Canellas, L.P., Spaccini, R., Piccolo, A., Dobbss, L.B., Okorokova-Facanha, A.L., Santos, G.D.A., Olivares, F.L., Facanha, A.R.	Relationships between chemical characteristics and root growth promotion of humic acids isolated from Brazilian oxisols	2009	Soil Science	174	11	611	620
14	99	Bandiera, M., Mosca, G., Vamerali, T.	Humic acids affect root characteristics of fodder radish (Raphanus sativus L. var. oleiformis Pers.) in metal-polluted wastes	2009	Desalination	246	1-3	78	91
15	112	Nikbakht, A., Kafi, M., Babalar, M., Xia, Y.P., Luo, A., Etemadi, N.-A.	Effect of humic acid on plant growth, nutrient uptake, and postharvest life of gerbera	2008	Journal of Plant Nutrition	31	12	2155	2167
16	126	Eyheraguibel, B., Silvestre, J., Morard, P.	Effects of humic substances derived from organic waste enhancement on the growth and mineral nutrition of maize	2008	Bioresource Technology	99	10	4206	4212
17	127	De Santiago, A., Quintero, J.M., Carmona, E., Delgado, A.	Humic substances increase the effectiveness of iron sulfate and Vivianite preventing iron chlorosis in white lupin	2008	Biology and Fertility of Soils	44	6	875	883
18	128	Marino, G., Francioso, O., Carletti, P., Nardi, S., Gessa, C.	Mineral content and root respiration of in vitro grown kiwifruit plantlets treated with two humic fractions	2008	Journal of Plant Nutrition	31	6	1074	1090
19	132	Clapp, C.E., Shenker, M., Hayes, M.H.B., Liu, R., Cline, V.W., Palazzo, A.J., Chen, Y.	Microsystems for rapid evaluation of plant growth response to organic amendments	2008	Soil Science	173	5	342	349
20	141	Willis, J.M., Hester, M.W.	Evaluation of enhanced Panicum amarum establishment through fragment plantings and humic acid amendment	2008	Journal of Coastal Research	24	2 SUPPL.B	263	268
21	157	Zandonadi, D.B., Canellas, L.P., Facanha, A.R.	Indolacetic and humic acids induce lateral root development through a concerted plasmalemma and tonoplast H+ pumps activation	2007	Planta	225	6	1583	1595

22	173	Arancon, N.Q., Edwards, Clive.A., Lee, S., Byrne, R.	Effects of humic acids from vermicomposts on plant growth	2006	European Journal of Soil Biology	42	SUPPL. 1	65	69
23	174	Khan, S., Cao, Q., Chen, B.-D., Zhu, Y.-G.	Humic acids increase the phytoavailability of Cd and Pb to wheat plants cultivated in freshly spiked, contaminated soil	2006	Journal of Soils and Sediments	6	4	236	242
24	196	Gryndler, M., Hrselova, H., Sudova, R., Gryndlerova¡, H., Rezacova, V., Merhautova, V.	Hyphal growth and mycorrhiza formation by the arbuscular mycorrhizal fungus Glomus claroideum BEG 23 is stimulated by humic substances	2005	Mycorrhiza	15	7	483	488
25	216	Turkmen, O., Dursun, A., Turan, M., Erdinc, C.	Calcium and humic acid affect seed germination, growth, and nutrient content of tomato (Lycopersicon esculentum L.) seedlings under saline soil conditions	2004	Acta Agriculturae Scandinavica Section B: Soil and Plant Science	54	3	168	174
26	226	Arancon, N.Q., Lee, S., Edwards, C.A., Atiyeh, R.	Effects of humic acids derived from cattle, food and paper-waste vermicomposts on growth of greenhouse plants	2003	Pedobiologia	47	5-6	741	744
27	242	Sharif, M., Khattak, R.A., Sarir, M.S.	Effect of different levels of lignitic coal derived humic acid on growth of maize plants	2002	Communications in Soil Science and Plant Analysis	33	19-20	3567	3580
28	245	Badora, A.	Bioaccumulation of Al, Mn, Zn and Cd in Pea Plants (Pisum sativum L.) Against a Background of Unconventional Binding Agents	2002	Polish Journal of Environmental Studies	11	2	109	116
29	252	Atiyeh, R.M., Lee, S., Edwards, C.A., Arancon, N.Q., Metzger, J.D.	The influence of humic acids derived from earthworm-processed organic wastes on plant growth	2002	Bioresource Technology	84	1	7	14
30	276	Bidegain, R.A., Kaemmerer, M., Guiresse, M., Hafidi, M., Rey, F., Morard, P., Revel, J.C.	Effects of humic substances from composted or chemically decomposed poplar sawdust on mineral nutrition of ryegrass	2000	Journal of Agricultural Science	134	3	259	267
31	297	Liu, C., Cooper, R.J., Bowman, D.C.	Humic acid application affects photosynthesis, root development, and nutrient content of creeping bentgrass	1998	HortScience	33	6	1023	1025
32	300	Adani, F., Genevini, P., Zaccheo, P., Zocchi, G.	The effect of commercial humic acid on tomato plant growth and mineral nutrition	1998	Journal of Plant Nutrition	21	3	561	575
33	307	Loffredo, E., Senesi, N., D'Orazio, V.	Effects of humic acids and herbicides, and their combinations on the growth of tomato seedlings in hydroponics	1997	Journal of Plant Nutrition and Soil Science	160	5	455	461
34	325	Ayuso, M., Hernandez, T., Garcia, C., Pascual, J.A.	Stimulation of barley growth and nutrient absorption by humic substances originating from various organic materials	1996	Bioresource Technology	57	3	251	257

35	346	Lobartini, J.C., Tan, K.H., Rema, J.A., Gingle, A.R., Pape, C., Himmelsbach, D.S.	The geochemical nature and agricultural importance of commercial humic matter	1992	Science of the Total Environment	113	1-2	1	15
36	351	Senesi, N., Loffredo, E., Padovano, G.	Effects of humic acid-herbicide interactions on the growth of Pisum sativum in nutrient solution	1990	Plant and Soil	127	1	41	47
37	360	Cabrera, D., Young, S.D., Rowell, D.L.	The toxicity of cadmium to barley plants as affected by complex formation with humic acid	1988	Plant and Soil	105	2	195	204
38	376	Tan, K.H., Nopamornbodi, V.	Effect of different levels of humic acids on nutrient content and growth of corn (Zea mays L.)	1979	Plant and Soil	51	2	283	287
39	379	Vaughan, D., Linehan, D.J.	The growth of wheat plants in humic acid solutions under axenic conditions	1976	Plant and Soil	44	2	445	449