Universität Basel

Fakultät für Psychologie

Innovative Approach for Depression Treatment: Two Ways of Modifying the Gut-Microbiome to Treat Major Depressive Disorder

A cumulative dissertation submitted to the Faculty of Psychology, University of Basel, in partial fulfillment of the requirements for the degree of Doctor of Philosophy by

Jessica Patricia Kazimiera Doll

from Eriz, Bern

Basel, 2022

Universität
Basel

Fakultät für
Psychologie

Approved by the Faculty of Psychology at the request of

Professor Dr. med. Dominique J.-F. de Quervain

PD Dr. sc. Nat. André Schmidt

Date of the doctoral examination:

Dean of the Faculty of Psychology

Universität Basel

Fakultät für Psychologie

Declaration of Scientific Fairness

I, Jessica Patricia Kazimiera Doll, hereby declare that the present work was written independently without the help of third parties and without the use of any means other than those indicated. Sources used for help are marked as such. The manuscripts published or submitted for publication in journals were prepared in cooperation with the co-authors and were not published elsewhere by any of the participants, submitted for publication, or submitted to any other examination authority as a qualification paper. These are the following manuscripts:

- **Doll, J. P.**, Vázquez-Castellanos, J. F., Schaub, A. C., Schweinfurth, N., Kettelhack, C., Schneider, E., ... & Lang, U. E. (2022). Fecal Microbiota Transplantation (FMT) as an Adjunctive Therapy for Depression—Case Report. *Frontiers in Psychiatry*, *13*. DOI: 10.3389/fpsyt.2022.815422.
- Schaub, A. C., Schneider, E., Vazquez-Castellanos, J. F., Schweinfurth, N., Kettelhack, C., **Doll, J. P**., ... & Lang, U. E. (2022). Clinical, gut microbial and neural effects of a probiotic add-on therapy in depressed patients: a randomized controlled trial. *Translational Psychiatry*, *12*(1), 1-10.
  DOI: https://doi.org/10.1038/s41398-022-01977-z
- Schneider, E., **Doll, J. P. K.**, Schweinfurth, N., Kettelhack, C., Schaub, A. C., Yamanbaeva, G., ... & Schmidt, A. (2022). Effect of short-term, high-dose probiotic supplementation on cognition, related brain functions and BDNF in depressed patients: A secondary analysis of a randomized controlled trial. *Journal of Psychiatry and Neuroscience*. (Submitted for publication in June 2022).

Basel, 25.08.2022

Jessica Patricia Kazimiera Doll

Abstract

Depression is a debilitating disorder that affects people worldwide. However, at least one third of patients do not respond to existing therapies, and novel treatment methods are necessary. A promising new approach for treating depression targets the microbiota-gut-brain axis, which is linked to physiological, behavioral, and cognitive functions affected in major depressive disorder. Probiotics and fecal microbiota transplantation are two possible ways to modify the microbiota-gut-brain axis.

The first study in this thesis (Doll et al., 2022) presents two patients with diagnosed depression treated with fecal microbiota transplantation as an add-on therapy. Depressive symptoms decreased for both patients four weeks after the transplantation, with the effects lasting up to eight weeks in one patient. Gastrointestinal symptoms were reflected in microbiota changes and improved in one patient.

In the second study (Schaub et al., 2022), we examined the effect of a probiotic supplementation on depressive symptoms, the gut microbiota, and the brain in a randomized controlled trial. Depressive symptoms decreased over time in both groups, but the decrease was stronger in the probiotic group. Probiotics increased the abundance of the genus *Lactobacillus,* which was associated with decreased depressive symptoms in the probiotics group. Finally, putamen activation in response to neutral faces was significantly decreased after the probiotic intervention.

In the third study (Schneider et al., submitted), we investigated the effect of the probiotic supplementation on cognitive functions, brain-derived neurotrophic factor, and the brain. We found a significantly improved immediate recall for the probiotics group immediately after intervention and a trend for a time-group-interaction considering all timepoints. We also found a time-group-interaction in hippocampus activation during working memory processing, revealing a remediated hippocampus function in the probiotic group. However, we did not find significant changes of brain-derived neurotrophic factor.

The results of the three studies highlight the role of the microbiota-gut-brain axis in depression and emphasize the potential of adjuvant microbiota-related treatment approaches as accessible, pragmatic, and non-stigmatizing therapies for depression. Nonetheless, the safety issues of fecal microbiota transplantation and the modest sample size of our studies imply that large-scale studies are needed to better understand the underlying mechanisms of depression and the microbiota-gut-brain axis and to replicate and validate our results.

Acknowledgements

During my doctorate, I had the honor of being part of an experienced, multidisciplinary, and supporting environment and working together with excellent researchers to whom I would like to express my gratitude.

First, I would like to thank my two supervisors, Prof. Dr. Dominique de Quervain and PD Dr. André Schmidt for supporting me and the microbiota-gut-brain project throughout this entire time. I would like to express my appreciation for Prof. Dr. Dominique de Quervain for giving me the opportunity to learn about scientific practice and investigate such an interesting topic, thus giving me the chance to grow as a scientist. Further, I would like to express my appreciation for PD Dr. André Schmidt, who supported and encouraged me during every process of the doctorate. He has given me the opportunity to grow in various ways and find my own way as a researcher and psychotherapist; I am very grateful for that. I would also like to express my gratitude to Prof. Dr. Undine Lang, who has accompanied and encouraged me during the journey of the Ph.D. and supported my path as a researcher and psychotherapist. I would also like to thank her for financing a significant part of my work.

Second, I would like to thank the entire translational psychiatry team, for creating a safe and inspiring environment to learn and work. In particular, I would like to thank M.Sc. Anna-Chiara Schaub, who has been there since the start of my Ph.D. With her deep interest in research, she has motivated me to explore the world of science. I would also like to thank Dr. Else Schneider, who has taught me much about how to move within the field of research, specifically as a woman. I would further like to thank Verena Limbach, M.Sc. Lukas Sempach and Dr. Gulnara Yamanbaeva for co-working with me on several occasions. During the doctoral studies, I started my psychotherapy training and also worked on a team of medical doctors, nurses, and other aspiring psychotherapists in a department for affective, stress, and sleep disorders. I would like to express my deep appreciation for the opportunity to learn from them and share my work with them. They have supported our research projects with motivation and were interested in bringing the treatment of depression to a new level. Specifically, I would like to thank Dr. Nina Schweinfurth-Keck for being my mentor and role model from day one. Furthermore, I would like to thank Dr. Timur Liwinski for sharing his deep knowledge and ideas on this topic with me.

Third, I thank Prof. Dr. Jeroen Raes and Dr. Jorge F. Vazquez-Castellanos for their strong effort regarding the microbiota analysis and the sharing of their knowledge with me.

Fourth, I thank the Gertrud Thalmann-Fonds for financially supporting my Ph.D. work.

Fifth, I would like to express my thanks and gratitude to my teachers and co-students of the person-centered approach training in psychotherapy. It is so wonderful to feel accepted and supported and through that being able to thrive. And it is delightful to see all these aspiring psychotherapists who dedicate their life to people in need.

Finally, I would like to thank the dear people around me as well as my cats, Jin and Jack, and my dog, Gili, who have been there throughout the whole time. Particularly, I thank my sister, Dominika Nufer, for inspiring and motivationg me, and my dear friend, Salimata Seck, for teaching me so much about humans and life, and for supporting me along my way. I want to thank my parents, Irena Roth and Bénédict Schmassmann, who raised me to become anything and anyone I want and have always supported and believed in me. I would like to thank Wolfgang Roth, who was unable to accompany me throughout life in physical matter, but who was and is always in my heart, giving me strength. Importantly, I would like to express my deep gratitude and appreciation for my beloved partner, M.Sc. Tobias Doll, who is always there for me, who always supports and encourages me. Thank you for standing by my side and doing life with me.

Table of Contents

Abbreviations

MDD	major depressive disorder
QoL	quality of life
GI	gastrointestinal
MGBA	microbiome-gut-brain axis
FMT	fecal microbiome transplantation
(r)CDI	(recurrent) clostridium difficile infection
IBD	irritable bowel disease
IBS	irritable bowel syndrome
ASD	autism spectrum disorder
WHO	World Health Organization
FAO	Food and Agriculture Organization
RCT	randomized controlled trial
BDNF	brain-derived neurotrophic factor
TAU	treatment as usual
PFC	prefrontal cortex
HPA	hypothalamus-pituitary-adrenal
PA	physical activity
IL	interleukin
MIF	macrophage inhibiting factor
CRP	C-reactive protein
VLMT	verbal learning memory test
SAEs	serious adverse events

The only person who is educated is the one who has learned how to learn and change.

- *Carl Rogers,* Freedom to Learn *(1969, p. 104)*

1. Introduction

Major depressive disorder (MDD) affects more than 264 million people worldwide (Collaborators, 2018), impacting the functioning and quality of life (QoL; (Ormel et al., 2019). Depressed mood or diminished interest in activities most of the day nearly every day for at least two weeks characterizes MDD. Importantly, depression is also accompanied by other symptoms such as cognitive symptoms (e.g., difficulty concentrating), feelings of worthlessness or hopelessness, thoughts of death or suicide, psychomotor agitation or retardation changes in appetite, fatigue or reduced energy, to name just a few (Organization, 2018). MDD is a leading cause of the global burden of disease and disability (Whiteford et al., 2013). It is estimated that mental illnesses account for 32.4% of years lived with disability globally, of which more than one-third is due to the burden of depression (Association, 2013; Coccurello, 2019; Lecrubier, 2006; Richardson & Adams, 2018; Silveira et al., 2013; Vigo et al., 2016). Lifetime prevalence for MDD is estimated to range between 10–20%, and the 1-year prevalence varies between 5–10%, affecting people of all genders, ages, and socioeconomic statuses (Kessler et al., 2003; Kessler et al., 2005). At its worst, depression may lead to suicidal ideation and suicidal behavior; an approximate risk rate of suicidal ideation and behavior during a depressive episode stands at 15% (Coryell & Young, 2005; Mann, 2002; Organization, 2018; Orsolini et al., 2020; Ponsoni et al., 2018). Currently, there are a number of effective treatment options for depression (Park & Zarate, 2019; Rush et al., 2006; Sinyor et al., 2010). However, despite advances in therapy options, about one-third of patients do not respond to treatment after two or more trials of antidepressant medication (Rush et al., 2006; Sinyor et al., 2010). It has become apparent that there is a crucial need for the development of new options.

Recently, the importance of the gut and biochemical signaling between the gastrointestinal (GI) and central nervous systems—referred to as the microbiota-gut-brain axis (MGBA)—has attracted significant interest in research (Cryan & Dinan, 2012; Dinan & Cryan, 2013; Dinan &

Cryan, 2017; Foster & McVey Neufeld, 2013; Lynch & Pedersen, 2016). Approximately 1 x 10^{13} to 1 x 10^{14} microorganisms inhabit the human GI tract and are referred to as "microbiota" (Cryan & Dinan, 2012). These microorganisms contain 150 times as many genes as our entire human genome (Gill et al., 2006; Qin et al., 2010). Taken together, all genomes of all these microorganisms are referred to as the microbiome (Cryan & Dinan, 2012). A human adult microbiota consists of over 1000 species (Qin et al., 2010) and 7000 strains (Ley et al., 2006). Two predominant bacterial phylotypes in the human gut microbiota are *Bacteroidetes* and *Firmicutes* (Eckburg et al., 2005). The colonization commences at birth when vaginal or cesarian delivery exposes the infant to the mother's microbiota (Grenham et al., 2011; Mackie et al., 1999). To date, three distinct enterotypes have been defined by their bacterial composition: *Bacteroides spp*., *Prevotella spp*., and *Ruminococcus spp*. (Arumugam et al., 2011).

It is well known that a balanced bacterial composition confers health benefits, and that a balance disruption (resulting in dysbiosis) prevents disease susceptibility (Cryan & O'Mahony, 2011). Due to the immense influence of gut bacteria on health and disease, research has focused over the past decades on the impact of the gut microbiome on the brain and behavior (Cryan & Dinan, 2012). The MGBA describes the bi-directional pathway between the gut and the brain (Collins & Bercik, 2009; Cryan & O'Mahony, 2011) and involves several modalities as communication routes between the gut and the brain, including the vagus nerve, the endocrine and immune system, and metabolites (see **Figure 1**; (Cryan & Dinan, 2012).

Several studies have linked the gut microbiome to depression (Cryan & Dinan, 2012; Dinan & Cryan, 2013; Foster & McVey Neufeld, 2013; Simpson et al., 2021; Winter et al., 2018). Moreover, the gut microbiota composition appears to be altered in depressed people (Barandouzi et al., 2020; Cheung et al., 2019; Jiang et al., 2015; Nikolova, Smith, et al., 2021), presenting a predominance of potentially harmful bacterial groups and/or a reduction in beneficial bacterial groups (Jiang et al., 2015). Such dysbiosis of the gut microbiome could be related to depressive symptoms (Valles-Colomer et al., 2019; Zheng et al., 2016). Together, these studies support the hypothesis that modifying the gut microbiome could decrease MDD symptoms.

There are various ways to modify the gut microbiome, such as the administration of prebiotics (Chudzik et al., 2021; Desmedt et al., 2019; Liu et al., 2019), probiotics (Chudzik et al., 2021; Liu et al., 2019), postbiotics (Chudzik et al., 2021), or fecal microbiota transplantation (FMT; (Kelly et al., 2016).

The first part of this thesis will introduce gut microbiota modification via FMT and the current state of FMT research both in general and specifically in psychiatry. At this point, the first research paper comprising **study A)** entitled "Fecal Microbiota Transplantation (FMT) as an Adjunctive Therapy for Depression-Case Report," will be presented. In this study, we investigated the effect of frozen oral FMT capsules on depressive symptoms, GI symptoms, and the gut microbiota in patients with depression. As we terminated the study preterminally for safety reasons, we reported the results of two patients who had already received the FMT. The results indicate that there might be a potential for FMT as an add-on therapy for depression. However, the case report also shows that much more ground research is needed to understand the underlying mechanisms of the gut microbiome and the MGBA in order to administer FMT safely to patients.

For this study, my contribution covers the collection, curation, analysis, and visualization of the data on depressive symptoms and subjective GI data and the writing of the manuscript.

The second part of the thesis will introduce a more straightforward, more accessible gut modification: probiotic supplementation. The focus will be on the effect of a high-dose, short-term probiotic add-on intervention for MDD and will be presented by two original **studies: B)** "Clinical, gut microbial and neural effects of a probiotic add-on therapy in depressed patients: a randomized controlled trial," and **C)** "Effect of short-term, high-dose probiotic supplementation on cognition, related brain functions and BDNF in depressive patients: A secondary analysis of a randomized controlled trial."

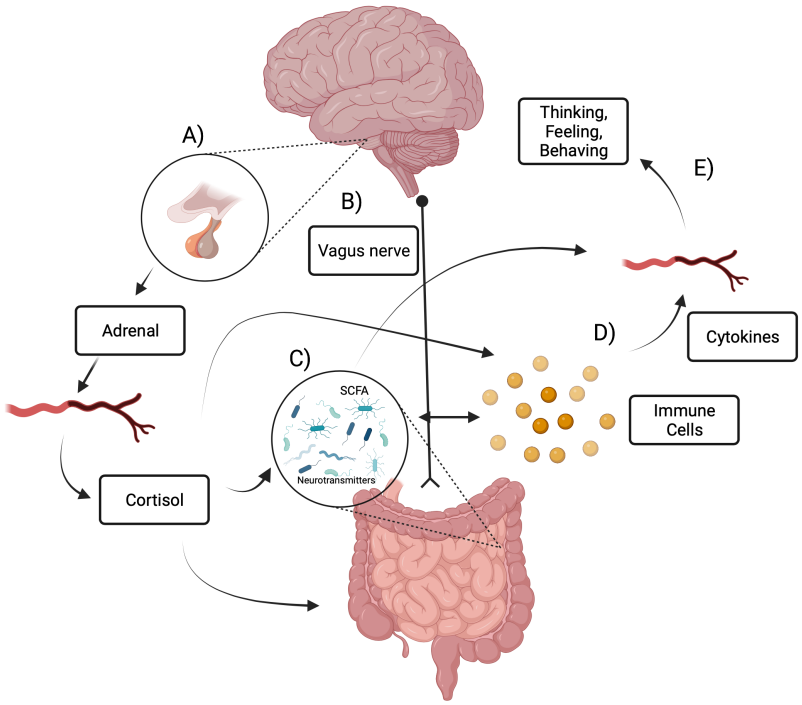
Study B) aimed at investigating the effects of a probiotic add-on treatment on depressive symptoms, gut microbiota, and brain structure and function. The results show that adjuvant probiotic treatment ameliorates depressive symptoms and produces changes in the gut microbiota and brain. My contribution covers the data collection and curation and reviewing the written manuscript.

In **study C)**, we analyzed the secondary data from **study B)** and investigated the effect of probiotic supplementation on cognitive symptoms, brain-derived neurotrophic factor (BDNF), and related brain areas. The results show that the probiotic supplementation enhances verbal episodic memory and directly affects neural mechanisms underlying impaired cognition in MDD.

My contribution to this study covers data collection and curation, cognition and BDNF data analysis, and some imaging data analysis, as well as the writing of the original manuscript.

Figure 1

The MGBA displayed with various communication pathways between the brain and the gut



Note. **A)** The endocrine system of the hypothalamus-pituitary-adrenal (HPA) axis. For example, stress leads to an increase in HPA axis activation and resulting increased cortisol levels. Cortisol can have an impact on the gut microbiome and gut permeability. **B)** Neural route of the vagus nerve. **C)** Neurotransmitters and short-chain-fatty acids (SCFA) in the gut, which can further impact the immune system, the brain and behavior. **D)** The immune system, which can be influenced by the gut microbiota and alter circulating cytokine levels, for example. **E)** Cytokines can influence the brain and thus have an impact on thinking, feeling, and behaving. Figure created in BioRender.com, based on Cryan & Dinan (2012).

2. FMT to modify the gut microbiota

FMT intends to introduce a beneficial microbial gut community by transferring intestinal microbiota from a healthy individual to the GI tract of another individual to cure a specific disease. To date, FMT has proven to be an effective treatment for recurrent *Clostridium difficile infection* (*rCDI*; (Kao et al., 2017; Lee et al., 2016). There is also potential for FMT in the treatment of inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and idiopathic constipation (Aroniadis & Brandt, 2013); however, further studies are needed to clarify the exact effects and mechanisms of FMT (Aroniadis et al., 2019; Xu et al., 2019).

FMT has mainly been studied in GI disorders; however, FMT might also be of use in psychiatry. For example, a significant number of children with autism spectrum disorder (ASD) experience GI symptoms compared to control groups, with an estimated odds ratio of 4.42 (McElhanon et al., 2014). Further, various studies have reported irregular gut microbiota in children with autism; thus, the hypothesis emerged that FMT might have the potential to ameliorate autism-related symptoms by modifying the gut microbiome (De Angelis et al., 2013; Kang et al., 2019; Son et al., 2015). Kang et al. (2017, 2019) reported significantly improved autism-related symptoms and overall symptom severity after FMT in 18 patients with ASD and GI symptoms (Kang et al., 2017). The effects maintained at an eight-week follow-up and a re-evaluation two years after the intervention showed that ASD-related symptoms further improved after the end of treatment compared to baseline (Kang et al., 2019; Kang et al., 2017). Additionally, FMT improved GI symptoms observed for all sub-categories of the GI rating scale and daily stool records (Kang et al., 2019; Kang et al., 2017).

The high comorbidity between GI and psychiatric symptoms is also present in depression. Significantly, IBS and depression often co-occur, and this further suggests a close link between the brain and the gut (Kurokawa et al., 2018; Person & Keefer, 2021). Based on this premise, preclinical evidence has shown that adult germ-free rodents receiving fecal samples from patients with MDD showed increased depressive-like behavior compared to controls (Kelly et al., 2016; Knudsen et al., 2021; Zheng et al., 2016). Therefore, transplanting healthy fecal microbiota to patients with MDD could potentially ameliorate depressive symptoms.

2.1. Fecal Microbiota Transplantation (FMT) as an Adjunctive Therapy for Depression-Case Report

3. Probiotics to modify the gut microbiota

According to the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO), probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). Probiotics have been studied in relation to various diseases and disorders (Kim et al., 2019), and interest has been drawn to the potential of probiotics in psychiatry, with a particular focus on depression.

3.1. Probiotics as adjuvant treatment for depression: The effect on affective symptoms, the gut microbiota, and the brain

Despite growing interest in the role of probiotics, empirical data on its effects in patients with MDD are still relatively scarce. Randomized controlled trials (RCT) found improved self-reported depressive symptoms in patients with MDD after an eight-week probiotic supplementation (Akkasheh et al., 2016; Kazemi et al., 2019). However, another RCT (Romijn et al., 2017) and a recent meta-analysis (Nikolova, Cleare, et al., 2021) indicated that probiotics effectively reduce depressive symptoms only when administered in addition to antidepressants, but not when used as a stand-alone treatment. This is in line with preclinical evidence showing that antidepressant medication increased gut microbiota diversity and that certain bacteria such as *Ruminococcus flavefaciens* specifically eliminated the antidepressant effect of the antidepressant duloxetine on depressive-like behavior in mice (Lukic et al., 2019).

Few studies have explored probiotic effects on gut microbiota and brain functions in participants with depressive symptoms (e.g., IBS patients with depressive symptoms). While some studies could not find any probiotic-induced changes in gut microbiota (Chahwan et al., 2019; Pinto-Sanchez et al., 2017a; Reininghaus et al., 2020), others reported an increased abundance of *Ruminococcus gauvreauii* (Reininghaus et al., 2020), decreased abundance of *Bacteroides* (Ng et al., 2013), and increased microbial diversity measures such as evenness at the genus level (Ng et al., 2013). Neuroimaging studies in healthy subjects and patients with IBS reported decreased activation in resting-state networks (Bagga et al., 2019) and regions related to cognition (Tillisch et al., 2013) and emotion (Bagga et al., 2018; Pinto-Sanchez et al., 2017a). Pinto-Sanchez et al. (2017) found that reduced amygdala responses to fearful faces correlated with changes in depressive symptoms in patients with IBS after probiotic treatment (Pinto-Sanchez et al., 2017a),

postulating that neural mechanisms may underlie the effect of probiotic treatment on depressive symptoms.

3.1.1. Clinical, gut microbial and neural effects of a probiotic add-on therapy in depressed patients: a randomized controlled trial

3.2. Probiotics as adjuvant treatment for depression: Cognitive symptoms, brain-derived neurotrophic factor, and related brain functions

Probiotics may not only be a potential treatment option for affective symptoms of depression but might also improve several cognitive functions. Studies reported improved cognitive functions, including verbal episodic memory, in healthy subjects and patients with MDD, in patients with Alzheimer's disease, minimal hepatic encephalopathy, and fibromyalgia (Kim et al., 2021; Lv et al., 2021).

To date, only one study has investigated the probiotic effect on cognition in a depressed population: Rudzki et al. (2019) reported that a placebo-controlled supplementation of *Lactobacillus Plantarum* significantly improved verbal memory recall in depressed people (Rudzki et al., 2019). Nonetheless, animal studies including FMT underscore the causal role of the gut microbiota in cognitive performance. When gut microbiota from old rats were transplanted to young rats, the young rats showed impaired cognitive performance and had reduced expression of brain-derived neurotrophic factor (BDNF), which is linked to hippocampal neurogenesis (Egan et al., 2003; Li et al., 2020; Rossi et al., 2006) and memory (Miranda et al., 2019). By contrast, transplanting microbiota from young to old rats resulted in improved cognitive performance (Boehme et al., 2021). Such findings indicate that beneficial bacteria, such as probiotics, have the potential to alter cognition, eventually via BDNF and the hippocampal regions.

3.2.1. Effect of short-term, high-dose probiotic supplementation on cognition, related brain functions, and BDNF in depressive patients: A secondary analysis of a randomized controlled trial

4. Discussion

Although MDD affects a significant number of people worldwide and several therapeutic options are available, current treatment methods are insufficient to achieve the expected and needed antidepressant effect (Rush et al., 2006; Sinyor et al., 2010). Novel therapeutic strategies are crucial to treat depression with all its symptoms and pathologies successfully (see **Figure 2**, section **A)**). In the frame of this thesis, three studies aimed at studying the effect of modifying the gut microbiome and targeting the MGBA to ameliorate symptoms of depression. In **study A)**, we investigated the effect of oral frozen FMT on depressive and GI symptoms and changes in the gut microbiome. In **study B)**, we researched the effects of probiotic supplementation on clinical symptoms of depression, changes in the gut microbiota, and the brain structure and volume. **Study C)** targeted the effects of the same probiotic supplementation on cognitive symptoms of depression, specifically on episodic verbal memory, BDNF, and hippocampal changes.

In **study A)**, the FMT case study, we showed that depressive symptoms improved in two patients four weeks after the intake of oral frozen FMT capsules. The positive depression outcome did not last for both patients; patient 1, who improved on GI symptoms, presented moderate depression scores eight weeks after the FMT. By contrast, patient 2, who did not improve significantly on GI symptoms, remained within the range of mild depression eight weeks after the FMT.

This case report has certain limitations. The limited sample size of only two patients limits its generalizability. Moreover, the comorbidities of both patients could also be confounding factors, as the outcome could be influenced by the comorbidities or medication intake. Furthermore, the patients received FMT in addition to treatment as usual (TAU), which makes attributing effects solely to FMT impossible. Another limitation is that this case report contains no information regarding the patients' diets even though studies indicate that diet is associated with depression and influences the gut microbiome (Lang et al., 2015). Furthermore, the amount of stool transplanted may play a crucial role in the effects of FMT; a comparatively low dose of donor stool (8.25 g) used in this study. Lastly, we do not have information or an analysis of the donor stool. Nonetheless, the microbial resemblance of the donor and recipient may play an important role, and this should be taken into consideration in future research.

In **study B),** the second study included in this thesis, we tested the potential of a short-term, high-dose probiotic supplementation as an adjuvant treatment for depression in an RCT. We were able to show a stronger amelioration of depressive symptoms after probiotic supplementation compared to placebo. This is in line with previous results reporting a continued beneficial effect of probiotics on depressive symptoms in patients with IBS (Pinto-Sanchez et al., 2017b). Our results suggest that an adjuvant probiotic treatment improves depressive symptoms, maintains healthy enterotypes species richness, and successfully engrafts specific health-related bacterial taxa (e.g., *Lactobacillus*). On a neural level, the probiotics additionally alter negative biases and emotional valence to TAU for depression.

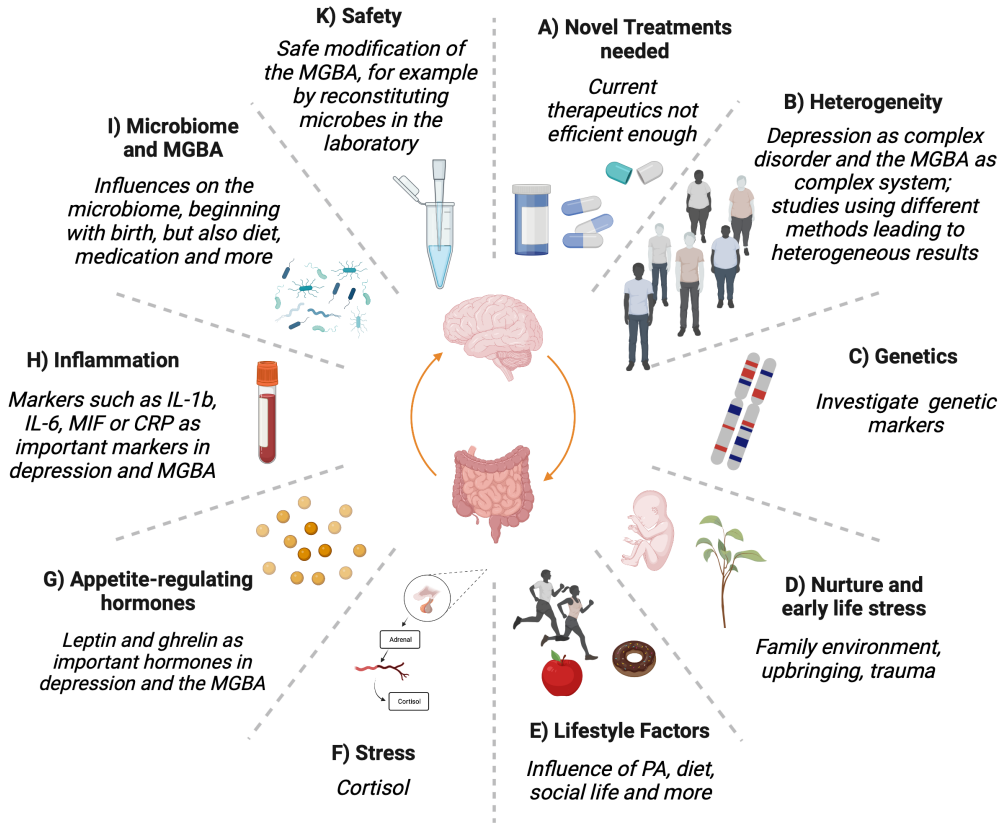
This study has several limitations. The sample size is relatively small, and compliance was imperfect even though the nursing personnel supplied the intervention product. Furthermore, the increased behavioral effect after eight weeks implies that changes in the brain structure and function might also be greater after eight weeks; however, we only have brain-related data from baseline and immediately after the intervention (four weeks after the start of the intervention); this is a limited time frame.

In the third study, **study C)**, we found improved immediate recall as measured by the verbal learning memory test (VLMT) after four weeks of probiotic supplementation. Our finding is in line with previous studies reporting a positive effect of probiotics on episodic memory (Ceccarelli et al., 2017; Rudzki et al., 2019; Xie & Prasad, 2020). We concluded that additional probiotic supplementation improves both affective symptoms (Schaub et al., 2022) and verbal episodic memory, which are highly relevant in MDD. Probiotic supplementation directly affects the neural mechanisms underlying cognitive impairments in depression by balancing the altered hippocampal function during the 2-back task.

Although we found improved cognitive performance due to the probiotic supplement, our study has some limitations. As this is a secondary analysis of the RCT of **study B)**, the same limitations apply to sample size, compliance, and brain-related imaging data.

Figure 2

Overview of the challenging aspects of depression and the MGBA and the implications for future studies



Note. **A)** Crucial need for novel treatments as current treatments are not efficient enough. **B)** Depression and the MGBA are complex systems. The differing methodologies used in studies are one reason for heterogeneous results and should be addressed in future studies. **C)** It is important to investigate genetic markers in depression and the MGBA to better understand depression. **D)** Nurture and early life stress are important factors in depression development and should be assessed in future studies. **E)** Lifestyle factors such as PA, diet, or social life are important factors in depression and may have an influence on the MGBA. **F)** Stress is associated with depression, and cortisol can have a significant impact on the gut microbiome. **G)** Appetite-regulating hormones leptin and ghrelin may play a crucial role in depression and the MGBA and could be important markers for depression treatment. **H)** Inflammation is tightly linked to the MGBA and involved in depression; it could be an important indicator to further develop efficient treatment options. **I)** The MGBA is a complex system involved in health and disease. Starting with birth, many factors can

influence the gut microbiome, leading to specific health or disease aspects, including depression. **K)** Safety issues are important indicators of the need of a better understanding of the underlying mechanisms of the gut microbiome and the MGBA. They should be improved to ensure safe gut microbiome modification. Figure created with BioRender.com.

Based on the work accomplished for this thesis and the current state of research, the potential of gut modification as an add-on treatment for depression becomes apparent. Nevertheless, the results are heterogeneous (see **Figure 2 B)**), and the exact underlying mechanisms are not yet fully understood. For example, some studies have found improvement in depressive symptoms and QoL after FMT (El-Salhy et al., 2020; Kurokawa et al., 2018); however, others found contrary results with QoL or with depressive symptoms not being affected by FMT (Aroniadis et al., 2019; El-Salhy et al., 2020). One major factor for heterogeneity in studies with FMT is the differing methodology used in these studies (Barbara & Ianiro, 2020), such as choosing one (super-)donor for the transplant (El-Salhy et al., 2020) or rather several donors (Aroniadis et al., 2019). Further methodological issues could be the FMT administration (e.g., oral capsule or colonoscopy) or type of formulation (e.g., frozen, dried, or fresh; (Barbara & Ianiro, 2020). Studies also present contrasting findings regarding the dose and frequency of FMT (Barbara & Ianiro, 2020; El-Salhy et al., 2020; Ianiro et al., 2019). It is possible, however, that the quantity might be less important than the quality. According to Barbara and Ianiro (2020), transplanting any healthy microbial biomass appears to be sufficiently efficient for CDI, regardless of the microbial composition of the donor or its resemblance to the donor. However, a favorable microbiome profile is required to achieve clinical success in other non-infectious chronic disorders such as ulcerative colitis (Barbara & Ianiro, 2020; Moayyedi et al., 2015). So far, it is uncertain which microbial profile would be the most favorable for treating depression. As previously found for IBS (Holvoet et al., 2021), FMT success might depend on the recipient's and donor's microbial composition before FMT and the recipient-donor-resemblance of the microbiota composition in the case of depression.

Compared to FMT, there is more research on the effect of probiotics on depressive symptoms; however, the findings are also heterogeneous, and we cannot conclude a definitive beneficial impact of probiotics on depressive symptoms.

Similar to FMT, differences often stem from varying methods such as sample characteristics, probiotic formula (e.g., single strain vs. multiple strain), or durations of interventions (Nadeem et al., 2019). Differing gut microbiota characteristics of the participants may cause further differences (Nadeem et al., 2019). Nonetheless, probiotics do seem to produce therapeutic effects for depressive symptoms and mood. Similar to our results, Huang et al. (2016) found positive effects of probiotics on mood in a group of depressed people in their meta-analysis (Huang et al., 2016). Moreover, they also found a positive effect on mood in healthy individuals. On the other hand, Ng et al. (2018) found the positive effect of probiotics in their meta-analysis to be significant only in a subgroup of people with pre-existing depressive symptoms (Ng et al., 2018), which indicates that certain subgroups might benefit from probiotics while others would not.

Probiotics have not only been shown to reduce depressive symptoms and improve mood, but they have also been shown to improve cognitive symptoms. In our **study C),** we found that probiotics improved verbal episodic memory. Several other studies have reported the positive effect of probiotics on cognition such as episodic memory (Ceccarelli et al., 2017; Rudzki et al., 2019; Xie & Prasad, 2020), global cognition (Ceccarelli et al., 2017; Den et al., 2020), spatial learning (Rezaei Asl et al., 2019), and executive functions and attention (Ceccarelli et al., 2017; Kim et al., 2021; Rudzki et al., 2019). The results on cognition also appear heterogeneous, as methods vary between studies and different tasks are used for measuring different cognitive domains (Ceccarelli et al., 2017; Den et al., 2020; Kelly et al., 2017; Lv et al., 2021; Marx et al., 2020; Reininghaus et al., 2018; Roman et al., 2018). It might be that the probiotic effects on cognition are domain-specific, causing heterogeneous results depending on the assessed cognitive domain. This could explain why we found dissimilar results across different cognitive domains relying on the neural mechanisms stimulated by probiotic supplementation.

On the neural level, our findings from **studies B)** and **C)** also support the claim of a beneficial effect of probiotics. In particular, the decrease of activation in the putamen during neutral face processing indicates a beneficial effect of probiotics on emotional information processing and can be interpreted as a shift in emotional valence of neutral faces: participants in the probiotics group perceived the neutral faces as more neutral after the intervention than before the intervention. Furthermore, the revealed hippocampal deactivation over time in the probiotics group is assumed to reflect the probiotics' beneficial effect on depression-related cognitive impairments. It is

postulated that disruptions of the hippocampal function in people with depression contribute to deficits in concentration and memory (MacQueen & Frodl, 2011; Smith et al., 2018; Videbech et al., 2001). As the hippocampus has strong neural connections to the prefrontal cortex (PFC; (Sampath et al., 2017) and belongs to the key structures that regulate the PFC function (Gurden et al., 2000; MacQueen & Frodl, 2011), hippocampal hyperactivity during working memory tasks in depression has been assumed to reflect difficulties in switching off self-referential default-mode processing (Harvey et al., 2005). Thus, the reduced hippocampal activation seen during the 2-back task performance after probiotic supplementation can be interpreted as an increased and remediated balance between the task-positive and default-mode networks during the working memory task, thus enhancing cognition. Eventually, our interpretation aligns with previous findings that report a positive impact of probiotics on the hippocampus (Tang et al., 2021) and that report effects of common antidepressants (Kaser et al., 2017; Planchez et al., 2020).

The heterogeneous results might also reflect the heterogeneity of depression as a highly complex disorder and the heterogeneity of GI disorders (displayed in **Figure 2** section **B)**).
A variety of symptoms characterize MDD, such as depressive mood, loss of pleasure, cognitive symptoms, neurovegetative symptoms, decreased energy levels, or disruption in appetite ((WHO), 2019/2021); moreover, a series of factors contribute to the development of MDD, such as genetics ((Howard et al., 2019), early life stress (LeMoult et al., 2020), nurture (Kendler et al., 2020), or lifestyle factors (Firth et al., 2020); see **Figure 2** section **C)**, **D)**, **E)**. The following discussion is an attempt to shed light on different aspects of depression and relate them to the involvement of the MGBA in the symptomatology of depression.

Moreover, several systems are involved in depression. To date, it is well established that the hypothalamus-pituitary-adrenal (HPA) axis and cortisol play a crucial role in acute and chronic stress reactions (Burke et al., 2005). Several studies show that the HPA axis is involved in depressive pathology, suggesting that cortisol is affected by current depression and is a risk factor for future depression (**Figure 2**, section **F);** Herbert, 2013). As described previously in **Figure 1 A)**, the HPA axis also plays a crucial role for the gut microbiota as cortisol may influence the gut permeability and the gut microbiota composition (Cryan & Dinan, 2012). Research has shown that cortisol is influenced by various factors, including physical activity (PA; (Beserra et al., 2018). PA belongs to lifestyle factors (**Figure 2 E)**) and might decrease cortisol levels in healthy people and

patients with MDD (Beserra et al., 2018). Additionally, PA is reported to have antidepressant effects and could be a protective factor against depression (Kandola et al., 2019; Schuch et al., 2018). However, PA is often reduced in depression, resulting in a more sedentary lifestyle with less physical exercise (Roshanaei-Moghaddam et al., 2009). Patients with MDD are often trapped in a vicious circle, as decreased levels of exercise due to low motivation and lack of energy are typical in depression, and decreased exercise may be a risk factor for depression (Roshanaei-Moghaddam et al., 2009). This is where the importance of the MGBA in MDD linked to PA comes into focus.

Preclinical findings show reduced PA as part of a depressive phenotype in mice, linked to the MGBA (Kelly et al., 2016; Zheng et al., 2016). These results further link the pathophysiological mechanisms of depression to the composition of the gut microbiota and reduced PA. Animal studies applying a probiotic supplementation reported that probiotics not only restored a depleted gut microbiome and reversed depressive-like behavior in animals—that is, reduced activity in the open field test (OFT; (Arseneault-Bréard et al., 2012; Desbonnet et al., 2008; Desbonnet et al., 2010)—but also that administering a single strain of probiotics to germ-free mice increased locomotor activity in the OFT (W. H. Liu et al., 2016; Y. W. Liu et al., 2016). Accordingly, altering the gut microbiota composition of patients with MDD could also improve patients' PA via physiological pathways.

Research conducted on the effect of probiotics on PA in patients with MDD is scarce. To date, there are some findings reporting changes in exercise capacities, mood, and stress levels in healthy athletes following probiotic supplementation (Dalton et al., 2019). Nevertheless, it remains unclear whether the underlying mechanisms linking beneficial effects of PA on MDD are mediated by the MGBA, and the exact nature of the relationship between probiotics and PA in the context of depression has yet to be understood.

Another modifiable lifestyle factor is diet, which can impact depressive symptoms and well-being (Lang et al., 2015). It would seem obvious that diet has a significant direct influence on the gut microbiota (Scott et al., 2013). Diet could therefore be associated with depressive symptoms via the MGBA due to changes in the gut microbiota (Jacka et al., 2017). Depressed patients often experience a significant change in appetite (Association, 2013; Coccurello, 2019). While diet may influence depression risk, depression itself may also have an impact on the choice of diet (Gomes et al., 2018; Jacka et al., 2015). Consequently, the appetite-regulating hormones leptin and ghrelin

might play an essential role in depressive symptomatology (**Figure 2 G)**). While the stomach mainly secretes the orexigenic hormone ghrelin (Kojima et al., 1999), it is the white adipose tissue that secretes the anorexigenic hormone leptin (Considine et al., 1996; Zhang et al., 1994). Nonetheless, reports of leptin and ghrelin in depression are scarce and inconsistent; they have mainly been reported in the context of energy balance and food intake (Ozsoy et al., 2014; Stone et al., 2020). Animal studies have shown associations between low leptin levels and stress and low leptin levels and depressive-like behavior, suggesting that leptin might have an antidepressant effect (Lu et al., 2006). A study including patients with depressive symptoms found elevated leptin levels with improvement in depressive symptoms after antidepressant treatment (Esel et al., 2005). However, other studies have shown decreased leptin levels with an improvement of depression (Rubin et al., 2002), no difference between patients with MDD and healthy controls, and no change in leptin levels after treatment (Ozsoy et al., 2014). Moreover, leptin showed inhibitory effects on the HPA axis (Stieg et al., 2015). The counterpart ghrelin appears to be increased by stress in animals (Kristenssson et al., 2006) and humans (Gecici et al., 2005; Yousufzai et al., 2018). Ghrelin has even been suggested to be named a stress hormone (Stone et al., 2020) with the potential to mediate stress via the HPA axis (Spencer et al., 2015). Studies investigating MDD in humans showed reduced (Barim et al., 2009) and elevated (Stone et al., 2020) ghrelin levels compared to healthy controls.

An emerging concept for understanding depression involves recognizing the immune system and the body's inflammatory response as systems involved in depression (**Figure 2 H)**). There is increasing evidence that MDD is related to a systemic immune activation, including abnormality in inflammatory markers (Beurel et al., 2020; Gibney & Drexhage, 2013; Müller, 2014). Proinflammatory cytokines, such as interleukin (IL)-1β and IL-6, are the main mediators of the inflammatory response and have been found to affect the brain (Haroon et al., 2012). They influence neurotransmission, neuroendocrine activity, and brain structure and functions, leading to potential cognitive, emotional, and behavioral changes in the body (Haroon et al., 2012). Multiple studies have shown altered levels of cytokines such as macrophage migration inhibiting factor (MIF), IL-6, and IL-1β, as well as acute phase proteins such as C-reactive protein (CRP) in patients with MDD (Beurel et al., 2020; Bloom & Al-Abed, 2014; Maes et al., 2009; Miller et al., 2009; Stewart et al., 2009).

Moreover, there appears to be a relation between inflammation and appetite-regulating hormones. Leptin was found to correlate positively with proinflammatory cytokines (e.g., IL-1β and IL-6), while ghrelin appears to downregulate proinflammatory cytokines (e.g., IL-1β and IL-6) and upregulate anti-inflammatory cytokines (e.g., IL-10; (Dixit et al., 2004; Mathur et al., 2020).

Furthermore, increased gene expression of the IL-1β, IL-6, and MIF genes was found in patients with MDD compared to healthy controls (Hepgul et al., 2013; Tsao et al., 2006). MIF is expressed in several parts of the brain and at every HPA axis level (Baugh & Donnelly, 2003; Bloom & Al-Abed, 2014). Circulating MIF levels are elevated in humans and animals suffering from stress or depressive symptoms, further suggesting a link between MIF and the pathology of depression (Baugh & Donnelly, 2003; Bloom & Al-Abed, 2014; Edwards et al., 2010). Additionally, psychological stress can often result in increased inflammation (Maydych, 2019), linking the inflammatory system to the HPA axis. Acute stressful events increase inflammatory activity and further neuropsychological changes that can modulate affective, behavioral, and cognitive activities, all of which are involved in MDD (Allen et al., 2014; Maydych, 2019; Slavich & Irwin, 2014). Chronic stress exposure causes immune system and endocrine system dysfunction that contributes to sustained low-grade inflammation, which also is involved in depressive symptomatology (Maydych, 2019; Rohleder, 2014). Similar to the HPA axis, the immune system is one of the crucial communication pathways between the brain and the gut. The gut microbiota or probiotic agents can alter cytokine levels, which can affect brain function (**Figure 1 D)** and **E)**; Cryan & Dinan, 2012). Clearly, the gut microbiome interacts with host immunity (Zheng et al., 2020), and an enhanced understanding of the complex interaction is necessary to further shed light on gut-microbiome-based therapies for depression.

Therefore, it would be extremely useful to further investigate the role of inflammation, HPA axis, appetite-regulating hormones, and lifestyle factors such as PA or diet in depressive symptoms and their relation to the MGBA. Such knowledge would make it possible to draw further conclusions about the underlying systems of depression and the gut microbiota, and the bi-directional communication between the brain and the gut. Consequently, the treatment of depression could become more specific and efficient. In 2020, Beurel et al. contributed to such investigations with their review on the bi-directional relationship between depression and inflammation (Beurel et al., 2020). They summarized graphically the symptoms of depression

associated with immunological alterations, in which appetite was associated with IL-6 and the microbiome; psychomotor retardation was also associated with IL-6, and also IL-1β; difficulty in concentration was associated with IL-6 and CRP; and much more (Beurel et al., 2020). Importantly, not all patients with MDD exhibit altered cytokine levels, and prior antidepressant intake might affect the cytokine production in some patients with MDD (Beurel et al., 2020). Furthermore, aberrant cytokine levels have been reported in other psychiatric disorders such as bipolar disorder or schizophrenia, suggesting common underlying immune mechanisms across various psychiatric disorders (Beurel et al., 2020; Goldsmith et al., 2016).

In an attempt to increase the knowledge on a more holistic level regarding depression and the involvement of the MGBA, we collected blood and saliva samples and measured the patients' PA both subjectively (by questionnaire) and objectively (by using an actigraph) in the RCT, which comprises also **study B)** and **study C)**. We are currently analyzing the data on inflammation markers ((IL)-1β, IL-6, CRP, and MIF), cortisol levels, appetite-regulating hormones (leptin and ghrelin), and PA measures. Our findings could present a substantial contribution to the understanding of the mechanisms involved in depression and how modification of the MGBA can improve depressive symptoms by acting on those mechanisms. Furthermore, many factors influence the gut microbiota, starting with birth (Wampach et al., 2018); in addition, diet, stress, and medication all can influence the gut microbiota throughout life, as indicated in **Figure 2 I)** (Hantsoo & Zemel, 2021; Jackson et al., 2018; Lukić et al., 2019; Maier & Typas, 2017; Molina-Torres et al., 2019; Scott et al., 2013). Ideally, such factors would be included in future studies.

Critically, the report of cases suffering from serious adverse events (SAEs) after FMT raises the importance of extensive donor screening and careful selection of patients receiving FMT, as indicated in **Figure 2 K)** (Barbara & Ianiro, 2020). To overcome these issues, more large-scale controlled clinical studies are needed with the aim of investigating gut microbiota modulation in depression; gaining knowledge of its underlying mechanisms, neuroactive potential, and beneficial and harmful microbes; and eventually reconstituting microbes in the laboratory. This would make safety control, traceability, and substantial FMT production possible (Barbara & Ianiro, 2020). Ideally, large-scale RCTs would make it possible to create subgroups of people with depression (e.g., based on depression severity, depressive symptoms, antidepressant medication, comorbidities, genetics, etc.) and investigate the effect of gut microbiota modification on a range

of depressive symptoms (e.g., affective symptoms, cognitive symptoms, psychomotor retardation, appetite, etc.) as well as substantial and sustainable brain-related changes. Utilizing this heterogeneity for the stratification of patients with MDD would enable a further step in precision psychiatry. Large-scale longitudinal studies assessing the above-mentioned factors involved in depression and the MGBA would further advance precise treatments for depression. By considering each individual's symptoms, genes, physiology, environment, and lifestyle, a high level of exactness would be achieved, eventually leading to personalized psychiatry. The resulting combined knowledge from probiotics and FMT studies could eventually lead to the translation of controlled and specific gut microbiota modification to clinical practice and, ultimately, to improve the well-being of individuals with depression.

5. Conclusion

MDD is characterized by various symptoms and several factors and systems contributing to the development and persistence of depression. Each person with depression presents a unique history and an individual range of symptoms. The MGBA has been postulated to play a crucial role in depression with several pathways as the communication route between the brain and the gut.

Our findings from **studies A)**, **B)**, and **C)** support the claim that the MGBA is important in MDD; moreover, the findings emphasize the potential of microbiota-related treatment approaches—especially probiotics—as accessible, non-stigmatizing, and holistic therapy that allows for the treatment of affective and cognitive symptoms in depression simultaneously. Importantly, our results should be validated and replicated in future studies. Our upcoming results on inflammation markers, stress markers, appetite-regulating hormones, and PA may bring further insight into the underlying mechanisms of depression and the role of the MGBA in MDD.

To overcome safety issues and improve treatment options by modifying the gut microbiota, more large-scale controlled clinical studies are needed. I am convinced that it is worth further pursuing this approach in order to improve the well-being of people with depression.

6. References

(WHO), W. H. O. (2019/2021). International Classification of Diseases, Eleventh Revision (ICD-11). In.

Akkasheh, G., Kashani-Poor, Z., Tajabadi-Ebrahimi, M., Jafari, P., Akbari, H., Taghizadeh, M., . . . Esmaillzadeh, A. (2016). Clinical and metabolic response to probiotic administration in patients with major depressive disorder: A randomized, double-blind, placebo-controlled trial. *Nutrition*, *32*(3), 315-320. https://doi.org/10.1016/j.nut.2015.09.003

Allen, A. P., Kennedy, P. J., Cryan, J. F., Dinan, T. G., & Clarke, G. (2014). Biological and psychological markers of stress in humans: focus on the Trier Social Stress Test. *Neurosci Biobehav Rev*, *38*, 94-124. https://doi.org/10.1016/j.neubiorev.2013.11.005

Aroniadis, O. C., & Brandt, L. J. (2013). Fecal microbiota transplantation: past, present and future. *Curr Opin Gastroenterol*, *29*(1), 79-84. https://doi.org/10.1097/MOG.0b013e32835a4b3e

Aroniadis, O. C., Brandt, L. J., Oneto, C., Feuerstadt, P., Sherman, A., Wolkoff, A. W., . . . Keller, M. J. (2019). Faecal microbiota transplantation for diarrhoea-predominant irritable bowel syndrome: a double-blind, randomised, placebo-controlled trial. *Lancet Gastroenterol Hepatol*, *4*(9), 675-685. https://doi.org/10.1016/S2468-1253(19)30198-0

Arseneault-Bréard, J., Rondeau, I., Gilbert, K., Girard, S. A., Tompkins, T. A., Godbout, R., & Rousseau, G. (2012). Combination of Lactobacillus helveticus R0052 and Bifidobacterium longum R0175 reduces post-myocardial infarction depression symptoms and restores intestinal permeability in a rat model. *Br J Nutr*, *107*(12), 1793-1799. https://doi.org/10.1017/S0007114511005137

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D. R., . . . Consortium, M. (2011). Enterotypes of the human gut microbiome. *Nature*, *473*(7346), 174-180. https://doi.org/10.1038/nature09944

Association, A. P. (2013). *Diagnostic and statistical manual of mental disorders (DSM-5®)*. American Psychiatric Pub.

Bagga, D., Aigner, C. S., Reichert, J. L., Cecchetto, C., Fischmeister, F. P. S., Holzer, P., . . . Schopf, V. (2019). Influence of 4-week multi-strain probiotic administration on resting-state functional connectivity in healthy volunteers. *European Journal of Nutrition*, *58*(5), 1821-1827. https://doi.org/10.1007/s00394-018-1732-z

Bagga, D., Reichert, J. L., Koschutnig, K., Aigner, C. S., Holzer, P., Koskinen, K., . . . Schopf, V. (2018). Probiotics drive gut microbiome triggering emotional brain signatures. *Gut Microbes*, *9*(6), 486-496. https://doi.org/10.1080/19490976.2018.1460015

Barandouzi, Z. A., Starkweather, A. R., Henderson, W. A., Gyamfi, A., & Cong, X. S. (2020). Altered Composition of Gut Microbiota in Depression: A Systematic Review. *Front Psychiatry*, *11*, 541. https://doi.org/10.3389/fpsyt.2020.00541

Barbara, G., & Ianiro, G. (2020). Faecal microbial transplantation in IBS: ready for prime time? *Gut*, *69*(5), 795-796.

Barim, A. O., Aydin, S., Colak, R., Dag, E., Deniz, O., & Sahin, I. (2009). Ghrelin, paraoxonase and arylesterase levels in depressive patients before and after citalopram treatment. *Clin Biochem*, *42*(10-11), 1076-1081. https://doi.org/10.1016/j.clinbiochem.2009.02.020

Baugh, J. A., & Donnelly, S. C. (2003). Macrophage migration inhibitory factor: a neuroendocrine modulator of chronic inflammation. *J Endocrinol*, *179*(1), 15-23. https://doi.org/10.1677/joe.0.1790015

Beserra, A. H. N., Kameda, P., Deslandes, A. C., Schuch, F. B., Laks, J., & Moraes, H. S. (2018). Can physical exercise modulate cortisol level in subjects with depression? A systematic review and meta-analysis. *Trends Psychiatry Psychother*, *40*(4), 360-368. https://doi.org/10.1590/2237-6089-2017-0155

Beurel, E., Toups, M., & Nemeroff, C. B. (2020). The Bidirectional Relationship of Depression and Inflammation: Double Trouble. *Neuron*, *107*(2), 234-256. https://doi.org/10.1016/j.neuron.2020.06.002

Bloom, J., & Al-Abed, Y. (2014). MIF: mood improving/inhibiting factor? *J Neuroinflammation*, *11*, 11. https://doi.org/10.1186/1742-2094-11-11

Boehme, M., Guzzetta, K. E., Bastiaanssen, T. F. S., van de Wouw, M., Moloney, G. M., Gual-Grau, A., . . . Cryan, J. F. (2021). Microbiota from young mice counteracts selective age-associated behavioral deficits. *Nature Aging*, *1*(8), 666-676. https://doi.org/10.1038/s43587-021-00093-9

Burke, H. M., Davis, M. C., Otte, C., & Mohr, D. C. (2005). Depression and cortisol responses to psychological stress: a meta-analysis. *Psychoneuroendocrinology*, *30*(9), 846-856. https://doi.org/10.1016/j.psyneuen.2005.02.010

Ceccarelli, G., Fratino, M., Selvaggi, C., Giustini, N., Serafino, S., Schietroma, I., . . . d'Ettorre, G. (2017). A pilot study on the effects of probiotic supplementation on

neuropsychological performance and microRNA-29a-c levels in antiretroviral-treated HIV-1-infected patients. *Brain Behav*, *7*(8), e00756. https://doi.org/10.1002/brb3.756

Chahwan, B., Kwan, S., Isik, A., van Hemert, S., Burke, C., & Roberts, L. (2019). Gut feelings: A randomised, triple-blind, placebo-controlled trial of probiotics for depressive symptoms. *Journal of Affective Disorders*, *253*, 317-326. https://doi.org/10.1016/j.jad.2019.04.097

Cheung, S. G., Goldenthal, A. R., Uhlemann, A. C., Mann, J. J., Miller, J. M., & Sublette, M. E. (2019). Systematic Review of Gut Microbiota and Major Depression. *Front Psychiatry*, *10*, 34. https://doi.org/10.3389/fpsyt.2019.00034

Chudzik, A., Orzyłowska, A., Rola, R., & Stanisz, G. J. (2021). Probiotics, Prebiotics and Postbiotics on Mitigation of Depression Symptoms: Modulation of the Brain-Gut-Microbiome Axis. *Biomolecules*, *11*(7). https://doi.org/10.3390/biom11071000

Coccurello, R. (2019). Anhedonia in depression symptomatology: Appetite dysregulation and defective brain reward processing. *Behav Brain Res*, *372*, 112041. https://doi.org/10.1016/j.bbr.2019.112041

Collaborators, G. D. a. I. I. a. P. (2018). Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet*, *392*(10159), 1789-1858. https://doi.org/10.1016/S0140-6736(18)32279-7

Collins, S. M., & Bercik, P. (2009). The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease. *Gastroenterology*, *136*(6), 2003-2014. https://doi.org/10.1053/j.gastro.2009.01.075

Considine, R. V., Considine, E. L., Williams, C. J., Hyde, T. M., & Caro, J. F. (1996). The hypothalamic leptin receptor in humans: identification of incidental sequence polymorphisms and absence of the db/db mouse and fa/fa rat mutations. *Diabetes*, *45*(7), 992-994. https://doi.org/10.2337/diab.45.7.992

Coryell, W., & Young, E. A. (2005). Clinical predictors of suicide in primary major depressive disorder. *J Clin Psychiatry*, *66*(4), 412-417. https://doi.org/10.4088/jcp.v66n0401

Cryan, J. F., & Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nature reviews neuroscience*, *13*(10), 701-712.

Cryan, J. F., & O'Mahony, S. M. (2011). The microbiome-gut-brain axis: from bowel to behavior. *Neurogastroenterol Motil*, *23*(3), 187-192. https://doi.org/10.1111/j.1365-2982.2010.01664.x

Dalton, A., Mermier, C., & Zuhl, M. (2019). Exercise influence on the microbiome-gut-brain axis. *Gut Microbes*, *10*(5), 555-568. https://doi.org/10.1080/19490976.2018.1562268

De Angelis, M., Piccolo, M., Vannini, L., Siragusa, S., De Giacomo, A., Serrazzanetti, D. I., . . . Francavilla, R. (2013). Fecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. *PloS one*, *8*(10), e76993.

Den, H., Dong, X., Chen, M., & Zou, Z. (2020). Efficacy of probiotics on cognition, and biomarkers of inflammation and oxidative stress in adults with Alzheimer's disease or mild cognitive impairment - a meta-analysis of randomized controlled trials. *Aging (Albany NY)*, *12*(4), 4010-4039. https://doi.org/10.18632/aging.102810

Desbonnet, L., Garrett, L., Clarke, G., Bienenstock, J., & Dinan, T. G. (2008). The probiotic Bifidobacteria infantis: An assessment of potential antidepressant properties in the rat. *J Psychiatr Res*, *43*(2), 164-174. https://doi.org/10.1016/j.jpsychires.2008.03.009

Desbonnet, L., Garrett, L., Clarke, G., Kiely, B., Cryan, J. F., & Dinan, T. G. (2010). Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. *Neuroscience*, *170*(4), 1179-1188. https://doi.org/10.1016/j.neuroscience.2010.08.005

Desmedt, O., Broers, V. J. V., Zamariola, G., Pachikian, B., Delzenne, N., & Luminet, O. (2019). Effects of prebiotics on affect and cognition in human intervention studies. *Nutr Rev*, *77*(2), 81-95. https://doi.org/10.1093/nutrit/nuy052

Dinan, T. G., & Cryan, J. F. (2013). Melancholic microbes: a link between gut microbiota and depression? *Neurogastroenterology & Motility*, *25*(9), 713-719.

Dinan, T. G., & Cryan, J. F. (2017). Gut instincts: microbiota as a key regulator of brain development, ageing and neurodegeneration. *The Journal of physiology*, *595*(2), 489-503.

Dixit, V. D., Schaffer, E. M., Pyle, R. S., Collins, G. D., Sakthivel, S. K., Palaniappan, R., . . . Taub, D. D. (2004). Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells. *J Clin Invest*, *114*(1), 57-66. https://doi.org/10.1172/JCI21134

Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., . . . Relman, D. A. (2005). Diversity of the human intestinal microbial flora. *Science*, *308*(5728), 1635-1638. https://doi.org/10.1126/science.1110591

Edwards, K. M., Bosch, J. A., Engeland, C. G., Cacioppo, J. T., & Marucha, P. T. (2010). Elevated macrophage migration inhibitory factor (MIF) is associated with depressive symptoms, blunted cortisol reactivity to acute stress, and lowered morning cortisol. *Brain Behav Immun*, *24*(7), 1202-1208. https://doi.org/10.1016/j.bbi.2010.03.011

Egan, M. F., Kojima, M., Callicott, J. H., Goldberg, T. E., Kolachana, B. S., Bertolino, A., . . . Weinberger, D. R. (2003). The BDNF val66met Polymorphism Affects Activity-Dependent Secretion of BDNF and Human Memory and Hippocampal Function. *Cell*, *112*(2), 257-269. https://doi.org/https://doi.org/10.1016/S0092-8674(03)00035-7

El-Salhy, M., Hatlebakk, J. G., Gilja, O. H., Kristoffersen, A. B., & Hausken, T. (2020). Efficacy of faecal microbiota transplantation for patients with irritable bowel syndrome in a randomised, double-blind, placebo-controlled study. *Gut*, *69*(5), 859-867.

Esel, E., Ozsoy, S., Tutus, A., Sofuoglu, S., Kartalci, S., Bayram, F., . . . Kula, M. (2005). Effects of antidepressant treatment and of gender on serum leptin levels in patients with major depression. *Prog Neuropsychopharmacol Biol Psychiatry*, *29*(4), 565-570. https://doi.org/10.1016/j.pnpbp.2005.01.009

FAO/WHO. (2001). Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria. In (pp. 1-34): FAO Rome.

Firth, J., Solmi, M., Wootton, R. E., Vancampfort, D., Schuch, F. B., Hoare, E., . . . Stubbs, B. (2020). A meta-review of "lifestyle psychiatry": the role of exercise, smoking, diet and sleep in the prevention and treatment of mental disorders. *World Psychiatry*, *19*(3), 360-380. https://doi.org/10.1002/wps.20773

Foster, J. A., & McVey Neufeld, K. A. (2013). Gut-brain axis: how the microbiome influences anxiety and depression. *Trends Neurosci*, *36*(5), 305-312. https://doi.org/10.1016/j.tins.2013.01.005

Gecici, O., Serteser, M., Emuel, M., & Demirel, R. (2005). Serum ghrelin and leptin levels in major depressive disorders. *Neurology Psychiatry and Brain Research*, *12*(1).

Gibney, S. M., & Drexhage, H. A. (2013). Evidence for a dysregulated immune system in the etiology of psychiatric disorders. *J Neuroimmune Pharmacol*, *8*(4), 900-920. https://doi.org/10.1007/s11481-013-9462-8

Gill, S. R., Pop, M., Deboy, R. T., Eckburg, P. B., Turnbaugh, P. J., Samuel, B. S., . . . Nelson, K. E. (2006). Metagenomic analysis of the human distal gut microbiome. *Science*, *312*(5778), 1355-1359. https://doi.org/10.1126/science.1124234

Goldsmith, D. R., Rapaport, M. H., & Miller, B. J. (2016). A meta-analysis of blood cytokine network alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder and depression. *Mol Psychiatry*, *21*(12), 1696-1709. https://doi.org/10.1038/mp.2016.3

Gomes, A. P., Oliveira Bierhals, I., Gonçalves Soares, A. L., Hellwig, N., Tomasi, E., Formoso Assunção, M. C., & Gonçalves, H. (2018). Interrelatioship between Diet Quality and Depressive Symptoms in Elderly. *J Nutr Health Aging*, *22*(3), 387-392. https://doi.org/10.1007/s12603-017-0963-7

Grenham, S., Clarke, G., Cryan, J. F., & Dinan, T. G. (2011). Brain-gut-microbe communication in health and disease. *Front Physiol*, *2*, 94. https://doi.org/10.3389/fphys.2011.00094

Gurden, H., Takita, M., & Jay, T. M. (2000). Essential role of D1 but not D2 receptors in the NMDA receptor-dependent long-term potentiation at hippocampal-prefrontal cortex synapses in vivo. *Journal of Neuroscience*, *20*(22), RC106-RC106.

Hantsoo, L., & Zemel, B. S. (2021). Stress gets into the belly: Early life stress and the gut microbiome. *Behavioural Brain Research*, *414*, 113474.

Haroon, E., Raison, C. L., & Miller, A. H. (2012). Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behavior. *Neuropsychopharmacology*, *37*(1), 137-162. https://doi.org/10.1038/npp.2011.205

Harvey, P.-O., Fossati, P., Pochon, J.-B., Levy, R., LeBastard, G., Lehéricy, S., . . . Dubois, B. (2005). Cognitive control and brain resources in major depression: An fMRI study using the n-back task. *NeuroImage*, *26*(3), 860-869. https://doi.org/https://doi.org/10.1016/j.neuroimage.2005.02.048

Hepgul, N., Cattaneo, A., Zunszain, P. A., & Pariante, C. M. (2013). Depression pathogenesis and treatment: what can we learn from blood mRNA expression? *BMC Med*, *11*, 28. https://doi.org/10.1186/1741-7015-11-28

Herbert, J. (2013). Cortisol and depression: three questions for psychiatry. *Psychol Med*, *43*(3), 449-469. https://doi.org/10.1017/S0033291712000955

Holvoet, T., Joossens, M., Vázquez-Castellanos, J. F., Christiaens, E., Heyerick, L., Boelens, J., . . . De Looze, D. (2021). Fecal Microbiota Transplantation Reduces Symptoms in

Some Patients With Irritable Bowel Syndrome With Predominant Abdominal Bloating: Short- and Long-term Results From a Placebo-Controlled Randomized Trial. *Gastroenterology*, *160*(1), 145-157.e148. https://doi.org/10.1053/j.gastro.2020.07.013

Howard, D. M., Adams, M. J., Clarke, T. K., Hafferty, J. D., Gibson, J., Shirali, M., . . . Consortium, M. D. D. W. G. o. t. P. G. (2019). Genome-wide meta-analysis of depression identifies 102 independent variants and highlights the importance of the prefrontal brain regions. *Nat Neurosci*, *22*(3), 343-352. https://doi.org/10.1038/s41593-018-0326-7

Huang, R., Wang, K., & Hu, J. (2016). Effect of Probiotics on Depression: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Nutrients*, *8*(8). https://doi.org/10.3390/nu8080483

Ianiro, G., Eusebi, L. H., Black, C. J., Gasbarrini, A., Cammarota, G., & Ford, A. C. (2019). Systematic review with meta-analysis: efficacy of faecal microbiota transplantation for the treatment of irritable bowel syndrome. *Aliment Pharmacol Ther*, *50*(3), 240-248. https://doi.org/10.1111/apt.15330

Jacka, F. N., Cherbuin, N., Anstey, K. J., & Butterworth, P. (2015). Does reverse causality explain the relationship between diet and depression? *J Affect Disord*, *175*, 248-250. https://doi.org/10.1016/j.jad.2015.01.007

Jacka, F. N., O'Neil, A., Opie, R., Itsiopoulos, C., Cotton, S., Mohebbi, M., . . . Berk, M. (2017). A randomised controlled trial of dietary improvement for adults with major depression (the 'SMILES' trial). *BMC Med*, *15*(1), 23. https://doi.org/10.1186/s12916-017-0791-y

Jackson, M. A., Verdi, S., Maxan, M.-E., Shin, C. M., Zierer, J., Bowyer, R. C., . . . Bell, J. T. (2018). Gut microbiota associations with common diseases and prescription medications in a population-based cohort. *Nature communications*, *9*(1), 1-8.

Jiang, H., Ling, Z., Zhang, Y., Mao, H., Ma, Z., Yin, Y., . . . Ruan, B. (2015). Altered fecal microbiota composition in patients with major depressive disorder. *Brain Behav Immun*, *48*, 186-194. https://doi.org/10.1016/j.bbi.2015.03.016

Kandola, A., Ashdown-Franks, G., Hendrikse, J., Sabiston, C. M., & Stubbs, B. (2019). Physical activity and depression: Towards understanding the antidepressant mechanisms of physical activity. *Neurosci Biobehav Rev*, *107*, 525-539. https://doi.org/10.1016/j.neubiorev.2019.09.040

Kang, D.-W., Adams, J. B., Coleman, D. M., Pollard, E. L., Maldonado, J., McDonough-Means, S., . . . Krajmalnik-Brown, R. (2019). Long-term benefit of Microbiota

Transfer Therapy on autism symptoms and gut microbiota. *Scientific reports*, *9*(1), 1-9.

Kang, D.-W., Adams, J. B., Gregory, A. C., Borody, T., Chittick, L., Fasano, A., . . . McDonough-Means, S. (2017). Microbiota transfer therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome*, *5*(1), 1-16.

Kao, D., Roach, B., Silva, M., Beck, P., Rioux, K., Kaplan, G. G., . . . Louie, T. (2017). Effect of Oral Capsule- vs Colonoscopy-Delivered Fecal Microbiota Transplantation on Recurrent Clostridium difficile Infection: A Randomized Clinical Trial. *JAMA*, *318*(20), 1985-1993. https://doi.org/10.1001/jama.2017.17077

Kaser, M., Deakin, J. B., Michael, A., Zapata, C., Bansal, R., Ryan, D., . . . Sahakian, B. J. (2017). Modafinil Improves Episodic Memory and Working Memory Cognition in Patients With Remitted Depression: A Double-Blind, Randomized, Placebo-Controlled Study. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *2*(2), 115-122. https://doi.org/https://doi.org/10.1016/j.bpsc.2016.11.009

Kazemi, A., Noorbala, A. A., Azam, K., Eskandari, M. H., & Djafarian, K. (2019). Effect of probiotic and prebiotic vs placebo on psychological outcomes in patients with major depressive disorder: A randomized clinical trial. *Clinical Nutrition*, *38*(2), 522-528. https://doi.org/10.1016/j.clnu.2018.04.010

Kelly, J. R., Allen, A. P., Temko, A., Hutch, W., Kennedy, P. J., Farid, N., . . . Dinan, T. G. (2017). Lost in translation? The potential psychobiotic Lactobacillus rhamnosus (JB-1) fails to modulate stress or cognitive performance in healthy male subjects. *Brain Behav Immun*, *61*, 50-59. https://doi.org/10.1016/j.bbi.2016.11.018

Kelly, J. R., Borre, Y., O' Brien, C., Patterson, E., El Aidy, S., Deane, J., . . . Dinan, T. G. (2016). Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. *J Psychiatr Res*, *82*, 109-118. https://doi.org/10.1016/j.jpsychires.2016.07.019

Kendler, K. S., Ohlsson, H., Sundquist, J., & Sundquist, K. (2020). The Rearing Environment and Risk for Major Depression: A Swedish National High-Risk Home-Reared and Adopted-Away Co-Sibling Control Study. *Am J Psychiatry*, *177*(5), 447-453. https://doi.org/10.1176/appi.ajp.2019.19090911

Kessler, R. C., Berglund, P., Demler, O., Jein, R., Koretz, D., Merikangas, K. R., . . . Wang, P. S. (2003). The epidemiology of Major Depressive Disorder. Results from the National Comorbidity Survey Replication. *JAMA*, *18*, 3095-3105.

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the national comorbidity survey replication. *Archives of General Psychiatry*, *62*, 593-602.

Kim, C. S., Cha, L., Sim, M., Jung, S., Chun, W. Y., Baik, H. W., & Shin, D. M. (2021). Probiotic Supplementation Improves Cognitive Function and Mood with Changes in Gut Microbiota in Community-Dwelling Older Adults: A Randomized, Double-Blind, Placebo-Controlled, Multicenter Trial. *J Gerontol A Biol Sci Med Sci*, *76*(1), 32-40. https://doi.org/10.1093/gerona/glaa090

Kim, S.-K., Guevarra, R. B., Kim, Y.-T., Kwon, J., Kim, H., Cho, J. H., . . . Lee, J.-H. (2019). Role of probiotics in human gut microbiome-associated diseases.

Knudsen, J. K., Michaelsen, T. Y., Bundgaard-Nielsen, C., Nielsen, R. E., Hjerrild, S., Leutscher, P., . . . Sørensen, S. (2021). Faecal microbiota transplantation from patients with depression or healthy individuals into rats modulates mood-related behaviour. *Sci Rep*, *11*(1), 21869. https://doi.org/10.1038/s41598-021-01248-9

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., & Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, *402*(6762), 656-660. https://doi.org/10.1038/45230

Kristenssson, E., Sundqvist, M., Astin, M., Kjerling, M., Mattsson, H., Dornonville de la Cour, C., . . . Lindström, E. (2006). Acute psychological stress raises plasma ghrelin in the rat. *Regul Pept*, *134*(2-3), 114-117. https://doi.org/10.1016/j.regpep.2006.02.003

Kurokawa, S., Kishimoto, T., Mizuno, S., Masaoka, T., Naganuma, M., Liang, K.-c., . . . Suda, W. (2018). The effect of fecal microbiota transplantation on psychiatric symptoms among patients with irritable bowel syndrome, functional diarrhea and functional constipation: an open-label observational study. *Journal of affective disorders*, *235*, 506-512.

Lang, U. E., Beglinger, C., Schweinfurth, N., Walter, M., & Borgwardt, S. (2015). Nutritional aspects of depression. *Cell Physiol Biochem*, *37*(3), 1029-1043. https://doi.org/10.1159/000430229

Lecrubier, Y. (2006). Physical components of depression and psychomotor retardation. *J Clin Psychiatry*, *67 Suppl 6*, 23-26.

Lee, C. H., Steiner, T., Petrof, E. O., Smieja, M., Roscoe, D., Nematallah, A., . . . Kim, P. T. (2016). Frozen vs Fresh Fecal Microbiota Transplantation and Clinical Resolution of Diarrhea in Patients With Recurrent Clostridium difficile Infection: A Randomized Clinical Trial. *JAMA*, *315*(2), 142-149. https://doi.org/10.1001/jama.2015.18098

LeMoult, J., Humphreys, K. L., Tracy, A., Hoffmeister, J. A., Ip, E., & Gotlib, I. H. (2020). Meta-analysis: Exposure to Early Life Stress and Risk for Depression in Childhood and Adolescence. *J Am Acad Child Adolesc Psychiatry*, *59*(7), 842-855. https://doi.org/10.1016/j.jaac.2019.10.011

Ley, R. E., Peterson, D. A., & Gordon, J. I. (2006). Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell*, *124*(4), 837-848. https://doi.org/10.1016/j.cell.2006.02.017

Li, Y., Ning, L., Yin, Y., Wang, R., Zhang, Z., Hao, L., . . . Zhang, C. (2020). Age-related shifts in gut microbiota contribute to cognitive decline in aged rats. *Aging*, *12*(9), 7801-7817. https://doi.org/10.18632/aging.103093

Liu, R. T., Walsh, R. F. L., & Sheehan, A. E. (2019). Prebiotics and probiotics for depression and anxiety: A systematic review and meta-analysis of controlled clinical trials. *Neurosci Biobehav Rev*, *102*, 13-23. https://doi.org/10.1016/j.neubiorev.2019.03.023

Liu, W. H., Chuang, H. L., Huang, Y. T., Wu, C. C., Chou, G. T., Wang, S., & Tsai, Y. C. (2016). Alteration of behavior and monoamine levels attributable to Lactobacillus plantarum PS128 in germ-free mice. *Behav Brain Res*, *298*(Pt B), 202-209. https://doi.org/10.1016/j.bbr.2015.10.046

Liu, Y. W., Liu, W. H., Wu, C. C., Juan, Y. C., Wu, Y. C., Tsai, H. P., . . . Tsai, Y. C. (2016). Psychotropic effects of Lactobacillus plantarum PS128 in early life-stressed and naïve adult mice. *Brain Res*, *1631*, 1-12. https://doi.org/10.1016/j.brainres.2015.11.018

Lu, X. Y., Kim, C. S., Frazer, A., & Zhang, W. (2006). Leptin: a potential novel antidepressant. *Proc Natl Acad Sci U S A*, *103*(5), 1593-1598. https://doi.org/10.1073/pnas.0508901103

Lukic, I., Getselter, D., Ziv, O., Oron, O., Reuveni, E., Koren, O., & Elliott, E. (2019). Antidepressants affect gut microbiota and Ruminococcus flavefaciens is able to abolish their effects on depressive-like behavior. *Translational Psychiatry*, *9*, Article 133. https://doi.org/10.1038/s41398-019-0466-x

Lukić, I., Getselter, D., Ziv, O., Oron, O., Reuveni, E., Koren, O., & Elliott, E. (2019). Antidepressants affect gut microbiota and Ruminococcus flavefaciens is able to abolish their effects on depressive-like behavior. *Translational psychiatry*, *9*(1), 1-16.

Lv, T., Ye, M., Luo, F., Hu, B., Wang, A., Chen, J., . . . Ding, Z. (2021). Probiotics treatment improves cognitive impairment in patients and animals: A systematic review and meta-analysis. *Neuroscience & Biobehavioral Reviews*, *120*, 159-172. https://doi.org/https://doi.org/10.1016/j.neubiorev.2020.10.027

Lynch, S. V., & Pedersen, O. (2016). The Human Intestinal Microbiome in Health and Disease. *N Engl J Med*, *375*(24), 2369-2379. https://doi.org/10.1056/NEJMra1600266

Mackie, R. I., Sghir, A., & Gaskins, H. R. (1999). Developmental microbial ecology of the neonatal gastrointestinal tract. *Am J Clin Nutr*, *69*(5), 1035S-1045S. https://doi.org/10.1093/ajcn/69.5.1035s

MacQueen, G., & Frodl, T. (2011). The hippocampus in major depression: evidence for the convergence of the bench and bedside in psychiatric research? *Molecular Psychiatry*, *16*(3), 252-264.

Maes, M., Yirmyia, R., Noraberg, J., Brene, S., Hibbeln, J., Perini, G., . . . Maj, M. (2009). The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. *Metab Brain Dis*, *24*(1), 27-53. https://doi.org/10.1007/s11011-008-9118-1

Maier, L., & Typas, A. (2017). Systematically investigating the impact of medication on the gut microbiome. *Current opinion in microbiology*, *39*, 128-135.

Mann, J. J. (2002). A current perspective of suicide and attempted suicide. *Ann Intern Med*, *136*(4), 302-311. https://doi.org/10.7326/0003-4819-136-4-200202190-00010

Marx, W., Scholey, A., Firth, J., D'Cunha, N. M., Lane, M., Hockey, M., . . . Jacka, F. (2020). Prebiotics, probiotics, fermented foods and cognitive outcomes: A meta-analysis of randomized controlled trials. *Neurosci Biobehav Rev*, *118*, 472-484. https://doi.org/10.1016/j.neubiorev.2020.07.036

Mathur, N., Mehdi, S. F., Anipindi, M., Aziz, M., Khan, S. A., Kondakindi, H., . . . Roth, J. (2020). Ghrelin as an Anti-Sepsis Peptide: Review. *Front Immunol*, *11*, 610363. https://doi.org/10.3389/fimmu.2020.610363

Maydych, V. (2019). The Interplay Between Stress, Inflammation, and Emotional Attention: Relevance for Depression. *Front Neurosci*, *13*, 384. https://doi.org/10.3389/fnins.2019.00384

McElhanon, B. O., McCracken, C., Karpen, S., & Sharp, W. G. (2014). Gastrointestinal symptoms in autism spectrum disorder: a meta-analysis. *Pediatrics*, *133*(5), 872-883.

Miller, A. H., Maletic, V., & Raison, C. L. (2009). Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry*, *65*(9), 732-741. https://doi.org/10.1016/j.biopsych.2008.11.029

Miranda, M., Morici, J. F., Zanoni, M. B., & Bekinschtein, P. (2019). Brain-Derived Neurotrophic Factor: A Key Molecule for Memory in the Healthy and the Pathological

Brain. *Frontiers in Cellular Neuroscience*, *13*, 363-363. https://doi.org/10.3389/fncel.2019.00363

Moayyedi, P., Surette, M. G., Kim, P. T., Libertucci, J., Wolfe, M., Onischi, C., . . . Lee, C. H. (2015). Fecal Microbiota Transplantation Induces Remission in Patients With Active Ulcerative Colitis in a Randomized Controlled Trial. *Gastroenterology*, *149*(1), 102-109.e106. https://doi.org/10.1053/j.gastro.2015.04.001

Molina-Torres, G., Rodriguez-Arrastia, M., Roman, P., Sanchez-Labraca, N., & Cardona, D. (2019). Stress and the gut microbiota-brain axis. *Behavioural pharmacology*, *30*(2), 187-200.

Müller, N. (2014). Immunology of major depression. *Neuroimmunomodulation*, *21*(2-3), 123-130. https://doi.org/10.1159/000356540

Nadeem, I., Rahman, M. Z., Ad-Dab'bagh, Y., & Akhtar, M. (2019). Effect of probiotic interventions on depressive symptoms: A narrative review evaluating systematic reviews. *Psychiatry Clin Neurosci*, *73*(4), 154-162. https://doi.org/10.1111/pcn.12804

Ng, Q. X., Peters, C., Ho, C. Y. X., Lim, D. Y., & Yeo, W. S. (2018). A meta-analysis of the use of probiotics to alleviate depressive symptoms. *J Affect Disord*, *228*, 13-19. https://doi.org/10.1016/j.jad.2017.11.063

Ng, S. C., Lam, E. F. C., Lam, T. T. Y., Chan, Y. W., Law, W. D., Tse, P. C. H., . . . Wu, J. C. Y. (2013). Effect of probiotic bacteria on the intestinal microbiota in irritable bowel syndrome. *Journal of Gastroenterology and Hepatology*, *28*(10), 1624-1631. https://doi.org/10.1111/jgh.12306

Nikolova, V. L., Cleare, A. J., Young, A. H., & Stone, J. M. (2021). Updated Review and Meta-Analysis of Probiotics for the Treatment of Clinical Depression: Adjunctive vs. Stand-Alone Treatment [Review]. *Journal of Clinical Medicine*, *10*(4), 14, Article 647. https://doi.org/10.3390/jcm10040647

Nikolova, V. L., Smith, M. R. B., Hall, L. J., Cleare, A. J., Stone, J. M., & Young, A. H. (2021). Perturbations in Gut Microbiota Composition in Psychiatric Disorders: A Review and Meta-analysis. *JAMA Psychiatry*. https://doi.org/10.1001/jamapsychiatry.2021.2573

Organization, W. H. (2018). Mental health atlas 2017. Geneva: World Health Organization; 2018. *World Health Organization. WHO MiNDbank. http://www.who.int/mental_health/mindbank/en*.

Ormel, J., Kessler, R. C., & Schoevers, R. (2019). Depression: more treatment but no drop in prevalence: how effective is treatment? And can we do better? *Curr Opin Psychiatry*, *32*(4), 348-354. https://doi.org/10.1097/YCO.0000000000000505

Orsolini, L., Latini, R., Pompili, M., Serafini, G., Volpe, U., Vellante, F., . . . De Berardis, D. (2020). Understanding the Complex of Suicide in Depression: from Research to Clinics. *Psychiatry Investig*, *17*(3), 207-221. https://doi.org/10.30773/pi.2019.0171

Ozsoy, S., Besirli, A., Abdulrezzak, U., & Basturk, M. (2014). Serum ghrelin and leptin levels in patients with depression and the effects of treatment. *Psychiatry Investig*, *11*(2), 167-172. https://doi.org/10.4306/pi.2014.11.2.167

Park, L. T., & Zarate, C. A. (2019). Depression in the Primary Care Setting. *N Engl J Med*, *380*(6), 559-568. https://doi.org/10.1056/NEJMcp1712493

Person, H., & Keefer, L. (2021). Psychological comorbidity in gastrointestinal diseases: Update on the brain-gut-microbiome axis. *Prog Neuropsychopharmacol Biol Psychiatry*, *107*, 110209. https://doi.org/10.1016/j.pnpbp.2020.110209

Pinto-Sanchez, M. I., Hall, G. B., Ghajar, K., Nardelli, A., Bolino, C., Lau, J. T., . . . Bercik, P. (2017a). Probiotic Bifidobacterium longum NCC3001 Reduces Depression Scores and Alters Brain Activity: A Pilot Study in Patients With Irritable Bowel Syndrome. *Gastroenterology*, *153*(2), 448-+. https://doi.org/10.1053/j.gastro.2017.05.003

Pinto-Sanchez, M. I., Hall, G. B., Ghajar, K., Nardelli, A., Bolino, C., Lau, J. T., . . . Bercik, P. (2017b). Probiotic Bifidobacterium longum NCC3001 Reduces Depression Scores and Alters Brain Activity: A Pilot Study in Patients With Irritable Bowel Syndrome. *Gastroenterology*, *153*(2), 448-459.e448. https://doi.org/10.1053/j.gastro.2017.05.003

Planchez, B., Surget, A., & Belzung, C. (2020). Adult hippocampal neurogenesis and antidepressants effects. *Current Opinion in Pharmacology*, *50*, 88-95. https://doi.org/https://doi.org/10.1016/j.coph.2019.11.009

Ponsoni, A., Branco, L. D., Cotrena, C., Shansis, F. M., Grassi-Oliveira, R., & Fonseca, R. P. (2018). Self-reported inhibition predicts history of suicide attempts in bipolar disorder and major depression. *Compr Psychiatry*, *82*, 89-94. https://doi.org/10.1016/j.comppsych.2018.01.011

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., . . . Consortium, M. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, *464*(7285), 59-65. https://doi.org/10.1038/nature08821

Reininghaus, E. Z., Platzer, M., Kohlhammer-Dohr, A., Hamm, C., Morkl, S., Bengesser, S. A., . . . Dalkner, N. (2020). PROVIT: Supplementary Probiotic Treatment and Vitamin

B7 in Depression-A Randomized Controlled Trial [Article]. *Nutrients*, *12*(11), 17, Article 3422. https://doi.org/10.3390/nu12113422

Reininghaus, E. Z., Wetzlmair, L. C., Fellendorf, F. T., Platzer, M., Queissner, R., Birner, A., . . . Dalkner, N. (2018). The Impact of Probiotic Supplements on Cognitive Parameters in Euthymic Individuals with Bipolar Disorder: A Pilot Study. *Neuropsychobiology*, 1-8. https://doi.org/10.1159/000492537

Rezaei Asl, Z., Sepehri, G., & Salami, M. (2019). Probiotic treatment improves the impaired spatial cognitive performance and restores synaptic plasticity in an animal model of Alzheimer's disease. *Behavioural Brain Research*, *376*, 112183. https://doi.org/https://doi.org/10.1016/j.bbr.2019.112183

Richardson, L., & Adams, S. (2018). Cognitive deficits in patients with depression. . *The Journal for Nurse Practitioners*, *14 (6)*, 437-443.

Rohleder, N. (2014). Stimulation of systemic low-grade inflammation by psychosocial stress. *Psychosom Med*, *76*(3), 181-189. https://doi.org/10.1097/PSY.0000000000000049

Roman, P., Estévez, A. F., Miras, A., Sánchez-Labraca, N., Cañadas, F., Vivas, A. B., & Cardona, D. (2018). A Pilot Randomized Controlled Trial to Explore Cognitive and Emotional Effects of Probiotics in Fibromyalgia. *Sci Rep*, *8*(1), 10965. https://doi.org/10.1038/s41598-018-29388-5

Romijn, A. R., Rucklidge, J. J., Kuijer, R. G., & Frampton, C. (2017). A double-blind, randomized, placebo-controlled trial of Lactobacillus helveticus and Bifidobacterium longum for the symptoms of depression. *Australian and New Zealand Journal of Psychiatry*, *51*(8), 810-821. https://doi.org/10.1177/0004867416686694

Roshanaei-Moghaddam, B., Katon, W. J., & Russo, J. (2009). The longitudinal effects of depression on physical activity. *Gen Hosp Psychiatry*, *31*(4), 306-315. https://doi.org/10.1016/j.genhosppsych.2009.04.002

Rossi, C., Angelucci, A., Costantin, L., Braschi, C., Mazzantini, M., Babbini, F., . . . Caleo, M. (2006). Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment [https://doi.org/10.1111/j.1460-9568.2006.05059.x]. *European Journal of Neuroscience*, *24*(7), 1850-1856. https://doi.org/https://doi.org/10.1111/j.1460-9568.2006.05059.x

Rubin, R. T., Rhodes, M. E., & Czambel, R. K. (2002). Sexual diergism of baseline plasma leptin and leptin suppression by arginine vasopressin in major depressives and

matched controls. *Psychiatry Res*, *113*(3), 255-268. https://doi.org/10.1016/s0165-1781(02)00263-9

Rudzki, L., Ostrowska, L., Pawlak, D., Małus, A., Pawlak, K., Waszkiewicz, N., & Szulc, A. (2019). Probiotic Lactobacillus Plantarum 299v decreases kynurenine concentration and improves cognitive functions in patients with major depression: A double-blind, randomized, placebo controlled study. *Psychoneuroendocrinology*, *100*, 213-222. https://doi.org/10.1016/j.psyneuen.2018.10.010

Rush, A. J., Trivedi, M. H., Wisniewski, S. R., Nierenberg, A. A., Stewart, J. W., Warden, D., . . . Fava, M. (2006). Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *Am J Psychiatry*, *163*(11), 1905-1917. https://doi.org/10.1176/ajp.2006.163.11.1905

Sampath, D., Sathyanesan, M., & Newton, S. S. (2017). Cognitive dysfunction in major depression and Alzheimer's disease is associated with hippocampal-prefrontal cortex dysconnectivity. *Neuropsychiatric disease and treatment*, *13*, 1509-1519. https://doi.org/10.2147/NDT.S136122

Schaub, A.-C., Schneider, E., Vazquez-Castellanos, J. F., Schweinfurth, N., Kettelhack, C., Doll, J. P., . . . Beglinger, C. (2022). Clinical, gut microbial and neural effects of a probiotic add-on therapy in depressed patients: a randomized controlled trial. *Translational Psychiatry*, *12*(1), 1-10.

Schuch, F. B., Vancampfort, D., Firth, J., Rosenbaum, S., Ward, P. B., Silva, E. S., . . . Stubbs, B. (2018). Physical Activity and Incident Depression: A Meta-Analysis of Prospective Cohort Studies. *Am J Psychiatry*, *175*(7), 631-648. https://doi.org/10.1176/appi.ajp.2018.17111194

Scott, K. P., Gratz, S. W., Sheridan, P. O., Flint, H. J., & Duncan, S. H. (2013). The influence of diet on the gut microbiota. *Pharmacological research*, *69*(1), 52-60.

Silveira, H., Moraes, H., Oliveira, N., Coutinho, E. S., Laks, J., & Deslandes, A. (2013). Physical exercise and clinically depressed patients: a systematic review and meta-analysis. *Neuropsychobiology*, *67*(2), 61-68. https://doi.org/10.1159/000345160

Simpson, C. A., Diaz-Arteche, C., Eliby, D., Schwartz, O. S., Simmons, J. G., & Cowan, C. S. M. (2021). The gut microbiota in anxiety and depression - A systematic review. *Clin Psychol Rev*, *83*, 101943. https://doi.org/10.1016/j.cpr.2020.101943

Sinyor, M., Schaffer, A., & Levitt, A. (2010). The sequenced treatment alternatives to relieve depression (STAR*D) trial: a review. *Can J Psychiatry*, *55*(3), 126-135. https://doi.org/10.1177/070674371005500303

Slavich, G. M., & Irwin, M. R. (2014). From stress to inflammation and major depressive disorder: a social signal transduction theory of depression. *Psychol Bull*, *140*(3), 774-815. https://doi.org/10.1037/a0035302

Smith, J., Browning, M., Conen, S., Smallman, R., Buchbjerg, J., Larsen, K. G., . . . Harmer, C. J. (2018). Vortioxetine reduces BOLD signal during performance of the N-back working memory task: a randomised neuroimaging trial in remitted depressed patients and healthy controls. *Mol Psychiatry*, *23*(5), 1127-1133. https://doi.org/10.1038/mp.2017.104

Son, J. S., Zheng, L. J., Rowehl, L. M., Tian, X., Zhang, Y., Zhu, W., . . . Robertson, C. E. (2015). Comparison of fecal microbiota in children with autism spectrum disorders and neurotypical siblings in the simons simplex collection. *PloS one*, *10*(10), e0137725.

Spencer, S. J., Emmerzaal, T. L., Kozicz, T., & Andrews, Z. B. (2015). Ghrelin's Role in the Hypothalamic-Pituitary-Adrenal Axis Stress Response: Implications for Mood Disorders. *Biol Psychiatry*, *78*(1), 19-27. https://doi.org/10.1016/j.biopsych.2014.10.021

Stewart, J. C., Rand, K. L., Muldoon, M. F., & Kamarck, T. W. (2009). A prospective evaluation of the directionality of the depression-inflammation relationship. *Brain Behav Immun*, *23*(7), 936-944. https://doi.org/10.1016/j.bbi.2009.04.011

Stieg, M. R., Sievers, C., Farr, O., Stalla, G. K., & Mantzoros, C. S. (2015). Leptin: A hormone linking activation of neuroendocrine axes with neuropathology. *Psychoneuroendocrinology*, *51*, 47-57. https://doi.org/10.1016/j.psyneuen.2014.09.004

Stone, L. A., Harmatz, E. S., & Goosens, K. A. (2020). Ghrelin as a Stress Hormone: Implications for Psychiatric Illness. *Biol Psychiatry*, *88*(7), 531-540. https://doi.org/10.1016/j.biopsych.2020.05.013

Tang, W., Meng, Z., Li, N., Liu, Y., Li, L., Chen, D., & Yang, Y. (2021). Roles of Gut Microbiota in the Regulation of Hippocampal Plasticity, Inflammation, and Hippocampus-Dependent Behaviors. *Frontiers in cellular and infection microbiology*, *10*, 611014-611014. https://doi.org/10.3389/fcimb.2020.611014

Tillisch, K., Labus, J., Kilpatrick, L., Jiang, Z., Stains, J., Ebrat, B., . . . Mayer, E. A. (2013). Consumption of Fermented Milk Product With Probiotic Modulates Brain Activity. *Gastroenterology*, *144*(7), 1394-U1136. https://doi.org/10.1053/j.gastro.2013.02.043

Tsao, C.-W., Lin, Y.-S., Chen, C.-C., Bai, C.-H., & Wu, S.-R. (2006). Cytokines and serotonin transporter in patients with major depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *30*(5), 899-905.

Valles-Colomer, M., Falony, G., Darzi, Y., Tigchelaar, E. F., Wang, J., Tito, R. Y., . . . Wijmenga, C. (2019). The neuroactive potential of the human gut microbiota in quality of life and depression. *Nature microbiology*, *4*(4), 623-632.

Videbech, P., Ravnkilde, B., Pedersen, A. R., Egander, A., Landbo, B., Rasmussen, N. A., . . . Rosenberg, R. (2001). The Danish PET/depression project: PET findings in patients with major depression. *Psychol Med*, *31*(7), 1147-1158. https://doi.org/10.1017/s0033291701004469

Vigo, D., Thornicroft, G., & Atun, R. (2016). Estimating the true global burden of mental illness. *Lancet Psychiatry*, *3*(2), 171-178. https://doi.org/10.1016/S2215-0366(15)00505-2

Wampach, L., Heintz-Buschart, A., Fritz, J. V., Ramiro-Garcia, J., Habier, J., Herold, M., . . . Wilmes, P. (2018). Birth mode is associated with earliest strain-conferred gut microbiome functions and immunostimulatory potential. *Nat Commun*, *9*(1), 5091. https://doi.org/10.1038/s41467-018-07631-x

Whiteford, H. A., Degenhardt, L., Rehm, J., Baxter, A. J., Ferrari, A. J., Erskine, H. E., . . . Vos, T. (2013). Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. *Lancet*, *382*(9904), 1575-1586. https://doi.org/10.1016/S0140-6736(13)61611-6

Winter, G., Hart, R. A., Charlesworth, R. P. G., & Sharpley, C. F. (2018). Gut microbiome and depression: what we know and what we need to know. *Rev Neurosci*, *29*(6), 629-643. https://doi.org/10.1515/revneuro-2017-0072

Xie, C., & Prasad, A. A. (2020). Probiotics Treatment Improves Hippocampal Dependent Cognition in a Rodent Model of Parkinson's Disease. *Microorganisms*, *8*(11), 1661.

Xu, D., Chen, V. L., Steiner, C. A., Berinstein, J. A., Eswaran, S., Waljee, A. K., . . . Owyang, C. (2019). Efficacy of Fecal Microbiota Transplantation in Irritable Bowel Syndrome: A Systematic Review and Meta-Analysis. *Am J Gastroenterol*, *114*(7), 1043-1050. https://doi.org/10.14309/ajg.0000000000000198

Yousufzai, M. I. U. A., Harmatz, E. S., Shah, M., Malik, M. O., & Goosens, K. A. (2018). Ghrelin is a persistent biomarker for chronic stress exposure in adolescent rats and humans. *Transl Psychiatry*, *8*(1), 74. https://doi.org/10.1038/s41398-018-0135-5

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., & Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature*, *372*(6505), 425-432. https://doi.org/10.1038/372425a0

Zheng, D., Liwinski, T., & Elinav, E. (2020). Interaction between microbiota and immunity in health and disease. *Cell Res*, *30*(6), 492-506. https://doi.org/10.1038/s41422-020-0332-7

Zheng, P., Zeng, B., Zhou, C., Liu, M., Fang, Z., Xu, X., . . . Xie, P. (2016). Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Mol Psychiatry*, *21*(6), 786-796. https://doi.org/10.1038/mp.2016.44

7. Appendix

The following pages present the supplementary information available for study A) and study B) as published. The supplementary information for study C) is included in the submitted version above.

7.1. Supplementary Information study A)

Supplementary Case Description

Patient 1 was a 53-year-old Caucasian female whose diagnosis comprised MDD and chronic constipation. According to the patient, her first depressive episode started in adolescence, with a suicide attempt later in life. She was first diagnosed with MDD in 2006 and reported no continuous period above two months without depressive symptoms since then. The patient had been hospitalized twice in her life. Depression is common in her intermediate family, with two male second-degree relatives who have suffered from it, one deceased from suicide. At the baseline assessment, the patient had been in stationary therapy for almost three months and treated for depression and constipation (for medical treatment details, see Supplementary Table 1). Nevertheless, depressive symptoms persisted and had increased in their severity. They included "feeling a great inner bleakness", "black thoughts" and "psychological pain".

Also stagnating at a deficient level was the patient's chronic constipation, which had never been alleviated during her life. Despite consulting a dietary coach, using various laxatives, and conducting enemas, defecation was only possible once every two weeks.

Patient 2 was a 58-year-old Caucasian woman with a diagnosis of recurrent MDD. According to the patient, she cannot remember her childhood. As a young woman, she was told that she had been sexually abused as a child. She cannot actively recall being abused; however, her life has been filled with psychological abuse within her family and depression ever since she can

remember. She was first diagnosed with depression in 1980 and has been hospitalized twice since then. A family history of depression is not known. After more than two months of in-patient treatment, the patient's symptoms persevered. Her mood was low, and she suffered from dissociation. During that time, whenever any negative emotions appeared, she would dissociate, switch to childlike behavior and sometimes suffer from a panic attack. The patient was medicated with antidepressants and benzodiazepines (for details, see Supplementary Table 2). At that point in time, the patient was suffering from a negative affective state and GI symptoms, such as flatulence and constipation.

Supplementary Methods

Inclusion Criteria:

1. Age ≥ 18, (2) Body Mass Index (BMI) 18.5 – 40 kg/m^2
2. Able to provide signed and dated informed consent
3. Hamilton Depression Rating Scale - 17(HAMD - 17) > 17 (1)
4. The patient receives treatment as usual (TAU) for depression
5. Inpatients and outpatients of the UPK

Exclusion Criteria:

1. Comorbid psychiatric disturbances such as substance abuse, bipolar disorder, schizophrenia and eating disorders led to the exclusion of participation
2. The same accounted for current medical conditions (i.e. infectious disease), dietary restrictions (also any deviation from UPK standard meals)
3. Recent use of medication aside from standard depression care (i.e. antibiotics)
4. Anticipated use of antibiotics in upcoming four weeks
5. Pregnancy

6. Inability to read and understand the participant's information and informed consent
7. Inability or unwillingness to swallow capsules
8. Active vomiting
9. Intestinal conditions: known or suspected megacolon and/or known small bowel ileus, major gastrointestinal surgery within three months before enrolment (except appendectomy or cholecystectomy), history of total colectomy or bariatric surgery
10. Concurrent intensive chemotherapy, radiation therapy or biological treatment for active malignancy
11. Life expectancy of < 6 months

Study Design

The initial study was conducted using a double-blind, placebo-controlled, randomized parallel-group design. The trial was approved by the local ethics committee (Ethikkommission Nordwest- und Zentralschweiz) and was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization Tripartite Guideline on Good Clinical Practice. All patients provided written informed consent. The study was registered at ClinicalTrials.gov prior to study start (NCT03281004). Measurements of depressive and gastrointestinal symptoms took place before the intervention (baseline) as well as four weeks after the intervention (post-intervention). During the four weeks after the intervention weekly assessments took place. A follow-up was conducted after eight weeks. For one of the patients, stool samples were available eight weeks after the intervention. For the other patient, stool samples at follow-up were not available.

While our RCT was running, the Food and Drug Administration (FDA) released a safety alert regarding FMT (https://www.fda.gov/safety/medical-product-safety-information/fecal-microbiota-transplantation-safety-alert-risk-serious-adverse-events-likely-due-transmission).

Serious adverse events (SAEs) were observed in six patients with *rCDI*, who received FMT capsules from the same stool bank as we did. Although the participants of our RCT did not report any SAEs, we decided to abort the study for safety reasons. Since the follow-up assessment was around the time that the initial RCT was aborted and the patient has had difficulties with the stool sampling method, we decided not to demand a follow-up sample from this patient. Both patients provided written informed consent

Randomization and Masking

Patients were randomly assigned in a 1:1 ratio to receive FMT or placebo capsules. The randomization schedule was produced by an independent researcher, held centrally and not divulged to anyone involved in the trial. A unique treatment number was used to identify each carton of the investigational medicinal product; FMT and placebo cartons were identical in appearance. After randomization, patients were allocated an investigational medicinal product pack in sequential treatment number order. Patients were unaware of whether they received the active product or the placebo and were informed about their allocation after study completion.

Intervention

Patients were administered 30 FMT or placebo capsules under the observation of a physician. The choice of 30 capsules was based on prior assessment amongst depressed patients, who expressed a preference for capsules and on existing experience of stool volumes delivered via colonoscopy for *CDI* and some dose-finding research, indicating that low-dose (30 capsules) and high dose (60 capsules) resulted in statistically similar resolution of GI symptoms in *CDI* patients (2). The effect of administering frozen fecal microbiota or FMT capsules appear to be similar to fresh fecal microbiota transplantation in *rCDI* treatment (3, 4). The drug substance contained frozen fecal microbiota, filtered to 330 microns, mixed with glycerol and saline. Each

30-capsule-dose consisted of approximately 8.25g of donor stool. It originated from a single donor within one collection window and was kept frozen either in a -80° Celsius freezer or on dry ice. The stool donor for patient 1 was not the same as for patient 2. The capsules are coated with an internal enteric coating, allowing for stability. Further, they are externally coated in an enteric polymer, allowing for targeted delivery of viable microbial communities to the colon. The placebo capsules were identical in appearance to active capsules but did not contain fecal samples. Placebo capsules contained an autoclaved solution of glycerol and saline, enclosed in an identical gelatin capsule as the active product, including the same enteric polymer coating. Here we report two patients who received the active product.

Microbiome Analysis

DNA extraction and sequencing. The fecal DNA was extracted following the protocol described in (5). Summarily, DNA was extracted from 150-200mg of the frozen samples using MagAttract PowerMicrobiome DNA/RNA KF kit (QIAGEN) following the manufacturer's instructions. The V4 region of 16S rRNA genes was amplified using the 515F /806R primer pair and purified using the QIAquick PCR Purification Kit. Sequencing was performed using the Illumina MiSeq platform (MiSeq Reagent Kit v2).

16s rRNA data processing. Amplicon data from the 16S rRNA gene was analyzed following the DADA2 pipeline specifications (Callahan et al., 2016) (Briefly, the first 30bp were removed, and the sequence length was set to 130bp and 200bp for the forward and reverse strands, respectively. The sequence error rate, dereplications, the inferred composition of the sample, and the chimaera removal were done using the DADA2 default parameters. The taxonomic assignation was done utilizing the DADA2 RDP implementation (R packages "dada2" function "assignTaxonomy") using as reference the rdp_train_set_16

(https://zenodo.org/record/801828#.Xe-PctF7mQc), similarly the amplicon sequence variant (ASV) annotation was done using the GTDB_bac120_arc122_ssu_r202_Species trainset (R packages "dada2" function "addSpecies"). The relative abundance was presented at the (ASV) level and summarized to the genus level.

Microbial load measurement by flow cytometry. The microbial load of the study cohort was measured as described previously (6). Briefly, 200-250 mg frozen (-80°C) fecal aliquots were diluted in saline solution (0.85% NaCl; VWR International, Germany) and filtered using a sterile syringe filter (pore size of 5 µm; Sartorius Stedim Biotech GmbH, Germany). Next, 1 mL of the microbial cell suspension obtained was stained with 1 µL SYBR Green I (1:100 dilution in DMSO; Thermo Fisher Scientific, Massachusetts, USA) and incubated for 15 min in the dark at 37°C. The flow cytometry analysis was performed using a C6 Accuri low cytometer (BD Biosciences, New Jersey, USA) based on Prest et al. (7). Fluorescence events were monitored using the FL1 533/30 nm and FL3 >670 nm optical detectors. The BD Accuri CFlow software was used to gate and separate the microbial fluorescence events on the FL1/FL3 density plot from the fecal sample background. A threshold value of 2000 was applied on the FL1 channel. Based on the exact weight of the aliquots analyzed, cell counts were converted to microbial loads per gram of fecal material.

Quantitative microbiome profiling**.** The quantitative microbiome profiling (QMP) matrix was built as described by Vandeputte and colleagues (6).

In brief, samples were downsized to even sampling depth, defined as the ratio between sampling size (16S rRNA gene copy number-corrected sequencing depth) and microbial load (the average total cell count per gram of frozen fecal material). 16S rRNA gene copy numbers were retrieved from the rRNA operon copy number database rrnDB33.

Fecal moisture content. The fecal moister content was determined as the percentage of mass loss after lyophilization from 0.2 g frozen aliquots of non-homogenized fecal material as previously done in (5).

Fecal calprotectin measurement. Fecal calprotectin concentrations were determined using the fCAL ELISA Kit (Bühlmann). The measurements were done on frozen fecal material (-80°C).

Diversity analysis. Diversity analysis was performed using the R statistical software (v3.6.3). The beta diversity analysis from the 16S rDNA amplicon sequence variant (ASV) data was estimated by the free statistical package R (v3.6.3). The Bray-Curtis index (library "Vegan", function "vegdist") was used to estimate the dissimilarities between samples in the QMP even sampling depth ASV table. The low frequent ASV (80% of zero data) were removed previous to the dissimilarity estimation. A distance-based redundancy analysis (dbRDA) (library "Vegan" function "capscale") was performed to reduce dimensionality in the taxonomic and functional distance matrix. The Permutational Multivariate Analysis of Variance Using Distance Matrices (ADONIS test) (library "vegan" function "adonis") clinical and metadata variables. The clinical meta-data variables were correlated into the ordination using the function envfit (library "vegan" function "envfit"). Data were hierarchically clustered using the ward.D method (library "stats" function "hclust") and visualized into a heatmap (library "gplots" function "heatmap.2"). The adonis and envfit p-values were adjusted using the Benjamini-Hochberg method (library "stats" function "p.adjust").

The observed richness, the Shannon and the Inverse Simpson index (library "phyloseq" function "estimate_richness") and Pielou's evenness (library "microbiome" function "evenness") were estimated at the genus level for each of the samples of the cohort.

Enterotyping. The 16s rRNA bacterial profiles were collapsed at the genus level and integrated along with the FGFP cohort as done in the previous work (5). The Identification of the enterotypes was accomplished with the Dirichlet-multinomial Model (DMM) approach in R (library "DirichletMultinomial" function "dmn").

Statistical analysis of the microbiome

The multivariable association between the clinical data and the bacterial taxa were estimated using a negative binomial mixed-effect model (MEM) (library "lme4" function "glmer.nb"), here the dependent variable was set as the abundance of the ASV, the fixed effect was the time variable, and the random effect part of the model was the subject ID. Similarly, the alpha diversity indices, as well as the clinical metadata variables, were model using a mixed-effect model (library "lme4" function "lmer"). The significance of the coefficients was measured by performing the type III ANOVA test over the residual sum of squares (library "car" function "Anova"). The differences between the subjects in the analysis were determined by applying DESeq2 into the ASV even sample depth matrix. The taxonomic summarization at the Phylum level was visualized into a barplot (library "ggplot").

The between-group comparisons of continuous variables were analyzed using Wilcoxon signed rank-sum test (library "stats", function "wilcox.test"). All p-values were adjusted using Benjamini-Hochberg's correction (library "stats", function "p.adjust").

The visualization of the bar plots and principal, boxplots, PcoA were done using the ggplot package.

Statistical analysis of mapped data

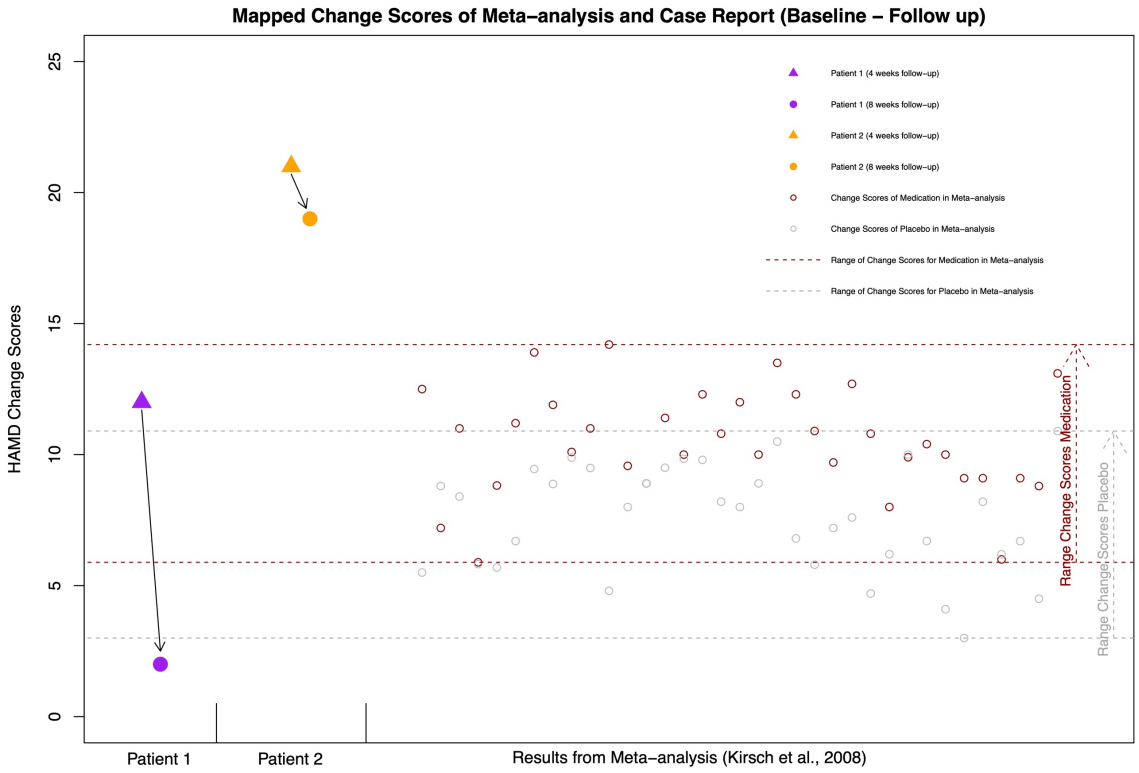
As the present results come from two cases and do not allow for statistical comparison as in RCTs, we mapped our HAMD change scores together with meta-analysis results from Kirsch

and colleagues (2008), which includes 35 trials investigating antidepressant treatment (8), to evaluate our findings in the field of depression treatment (Supplementary Figure 1). We calculated z-scores with RStudio (Version 1.4.1717) and acquired the corresponding p-values to investigate the probability that the change scores of the two cases fit into the (normal)distribution of the patients in the meta-analysis by Kirsch and colleagues (8).

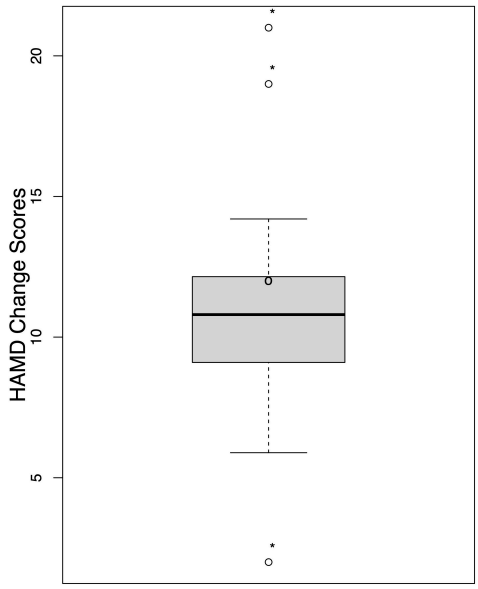
Supplementary Results

Results of our data mapped on data of a meta-analysis

Contrasting our FMT effects with effect sizes of antidepressant medications (8) revealed that patient 1 had a change score in line with that of antidepressant-medicated patients after four weeks; the change score of 12 appeared to fit into the change score distribution of medicated patients by Kirsch and colleagues (z = 0.76, p = 0.45). A relapse of symptoms followed (Supplementary Figure 1 and 2) and the eight-week change score of 2 differed from the change score distribution of medicated patients and was significantly lower (z = -4.2, p < 0.01). Compared to medicated patients in the meta-analysis, patient 2 greatly improved her depressive symptoms after four weeks of the intervention and remained stable until eight weeks follow up (Supplementary Figure 1 and 2). Four-week and eight-week change scores were higher than the change score distribution of the medicated patients in the meta-analysis by Kirsch and colleagues (four-week change score: z = 5.23, p < 0.01; eight-week change score: z = 4.24, p < 0.01), indicating greater improvement in our patient 2 compared to the medicated patients of the meta-analysis.



Supplementary Figure 1. Mapped change scores of patients 1 and 2 as well as change scores from Kirsch et al. (2008). Kirsch and colleagues' meta-analysis included 35 trials, investigating one of four antidepressants: Fluoxetine, Venlafaxine, Paroxetine and Nefazodone. As far as understandable from reported data, trials were of four, five, six- or eight-weeks duration (8). The range of change scores over all four medication treatments is indicted by the red dotted arrow, the range of overall placebo change scores with a grey dotted arrow. Purple symbols indicate patient 1, orange symbols patient 2. Triangle indicates the patients' change score four weeks post-FMT compared to baseline and the dots mark the change score eight weeks post-FMT compared to baseline. Black arrows illustrate the decrease of change score, which implies an aggravation of depressive symptoms.

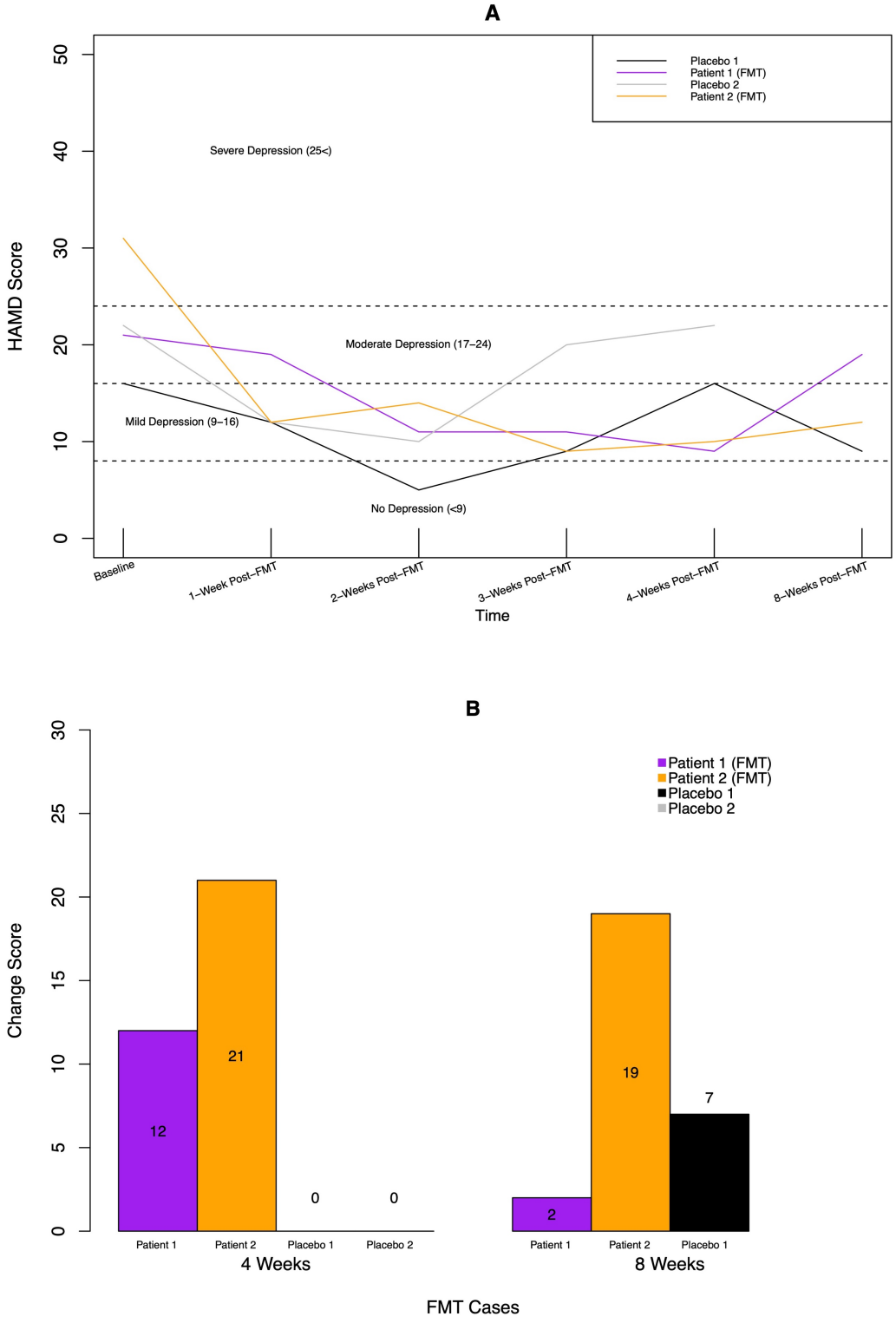


Supplementary Figure 2. Boxplot of HAMD change scores including medicated patients from meta-analysis by Kirsch et al. (2008) as well as the change scores of the two FMT cases (one point four weeks after FMT and one point eight weeks after FMT for each case). Three of the four change scores were outside of the distribution of medicated patients ($p < 0.01$).

Results of placebo participants

HAMD scores for both placebo participants improved in the two weeks after placebo-capsule intake. However, after the second week, symptom scores raised back up to the initial scores at baseline (placebo participant 1 from 16 at baseline back to 16 four weeks later at post-intervention and placebo participant 2 from 22 at baseline to 11 four weeks later at post-intervention). Eight weeks after the intervention, HAMD score for placebo participant 1 decreased to 9 points. HAMD score at follow up for placebo participant 2 is not available for two reasons: the depressive symptoms of this participant increased to a level as that she did not come back for post-intervention assessment. We then measured the HAMD score only by telephone. Additionally, at the timepoint of follow up measurement for placebo participant 2,

the study had already been terminated and as we then learned that she had received the placebo, we relinquished to do the follow-up assessment with her.



Supplementary Figure 3. A) HAMD scores for all 4 patients that had been included in the study at the time of premature termination of the study. Placebo patients are shown

in black and grey. HAMD score at follow-up for placebo participant 2 is not available for two reasons: the depressive symptoms of this participant increased to a level as that she did not come back for post-intervention assessment. We then measured the HAMD score only by telephone. Additionally, at the timepoint of follow-up measurement for placebo participant 2, the study had already been terminated and as we then learned that she had received the placebo, we relinquished to do the follow-up assessment with her. B) Change scores for both FMT patients (patient 1 and patient 2) and for both placebo participants (placebo 1 and placebo 2). As change scores were calculated by subtracting the score at post-intervention from the score at baseline, a higher change score indicates lower depressive symptoms score. Again, the follow-up score for placebo participant is not available.

Results of the bacterial taxa responding to FMT

Overall, microbiota composition in these two patients resembles that of the western population; *Feacalibacterium*, *Blautia*, and *Anaerostipes* were among the most abundant bacteria (Supplementary Figure 4 & B).

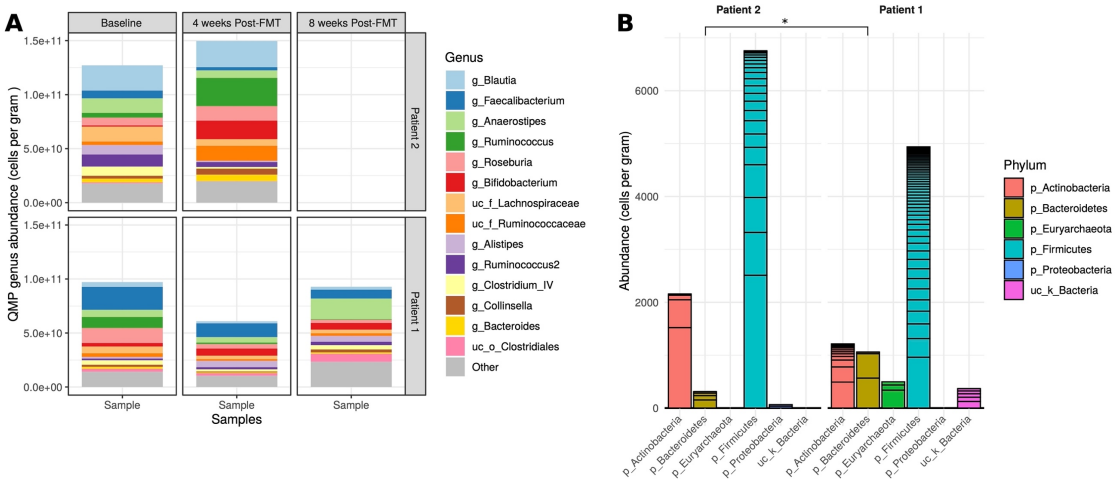
Over the whole dataset, microbial communities tended to cluster more according to participant identification number rather than time point, yet no significant effect was found for either variable (Supplementary Figure 5 B), Supplementary Table 3; Adonis adjusted $p = 0.226$).

The Deseq2 differential abundance analysis identified 46 different amplicon sequence variants (ASV) that were differentially enriched between the participants (Supplementary Figure 5 A) (DESeq2 adjusted $p < 0.05$). Patient 1 showed an increase of archaea and bacteria species abundant in low-transit-time bacterial communities, such as the phylum Euryarchaeota and the genus *Methanobrevibacter (5)* (Supplementary Figure 4 B and Supplementary Figure 5 A*)*; this

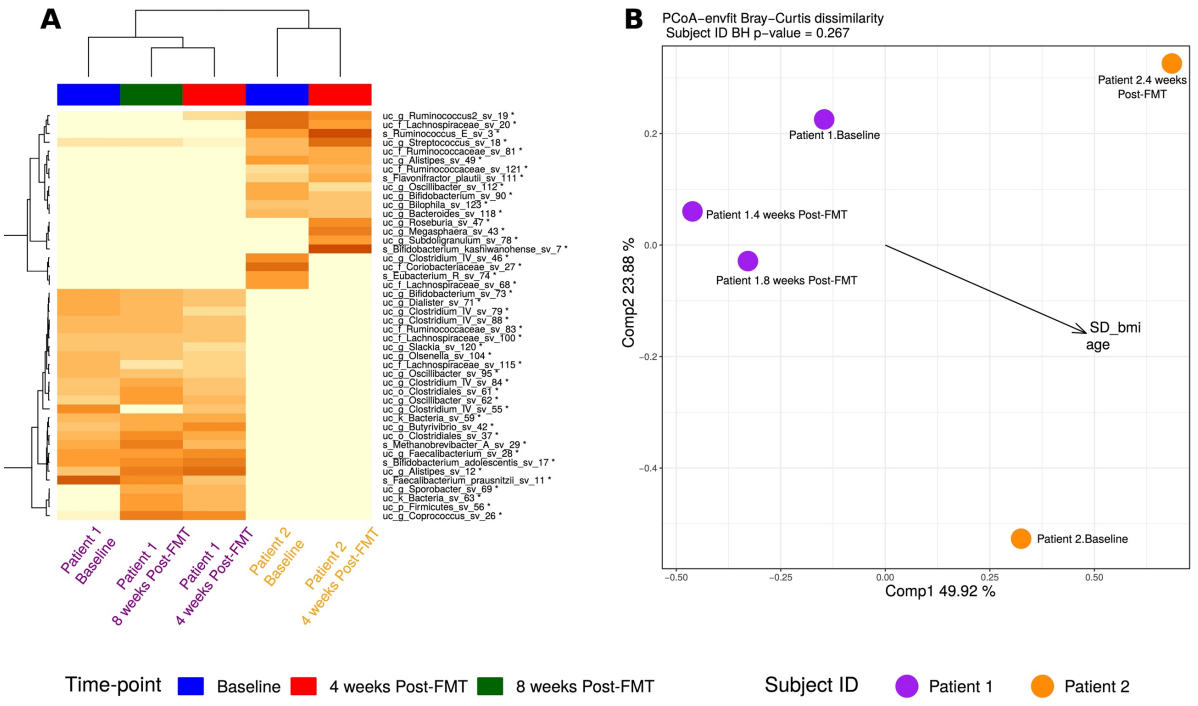
is congruent with the observed moisture level of the *Ruminocuccus* enterotype (9). Similarly, the patient showed an increase of ASV of the *genera Methanobrevibacter*, *Butyrivibrio*, *Sporobacter*, *Olsenella*, *Slackia, Faecalibacterium*, and *Dialister* (Supplementary Figure 5 A) (DESeq2 adjusted $p < 0.05$).

Patient 2 showed an increase of ASV of the species *Bifidobacterium kashiwanohense*, and of the genera *Ruminococcus* and *Roseburia*, *Eubacterium*, *Subdoligranulum*, *Flavonifractor*, *Bilophila* and, *Streptococcus* (Supplementary Figure 5 A).

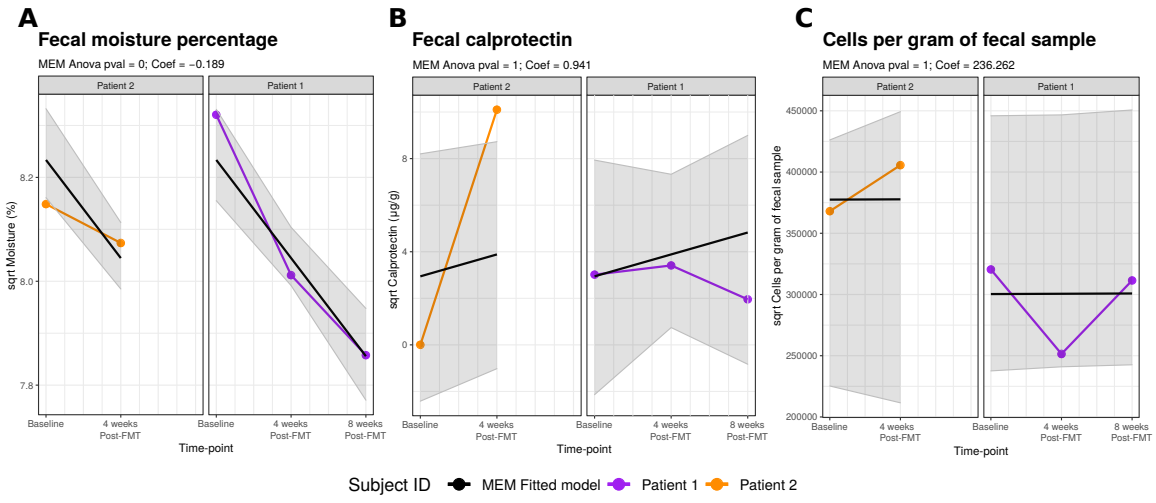
Besides differences, there were also similarities of FMT effects in these two patients. Common ASV changes were defined as such when both the continuous and discrete mixed-effect-model time coefficients were significant (adjusted $p < 0.1$) (Figure 3 E) and showed the same trend for both trial subjects. We found that the FMT increased the abundance of ASVs of the genera *Bifidobacterium*, *Blautia*, and the family Lachnospiraceae in both patients (Figure 3 E). Contrary, nine ASV of the genera *Eubacterium E, Coprococcus, Faecalibacterium, Butyricicoccus, Ruminococcus,* and *Bacteroides* and species from the Ruminococcaceae and *Lachnospiraceae* families such as *Lachnospira eligens*, were reduced (Figure 3 E).



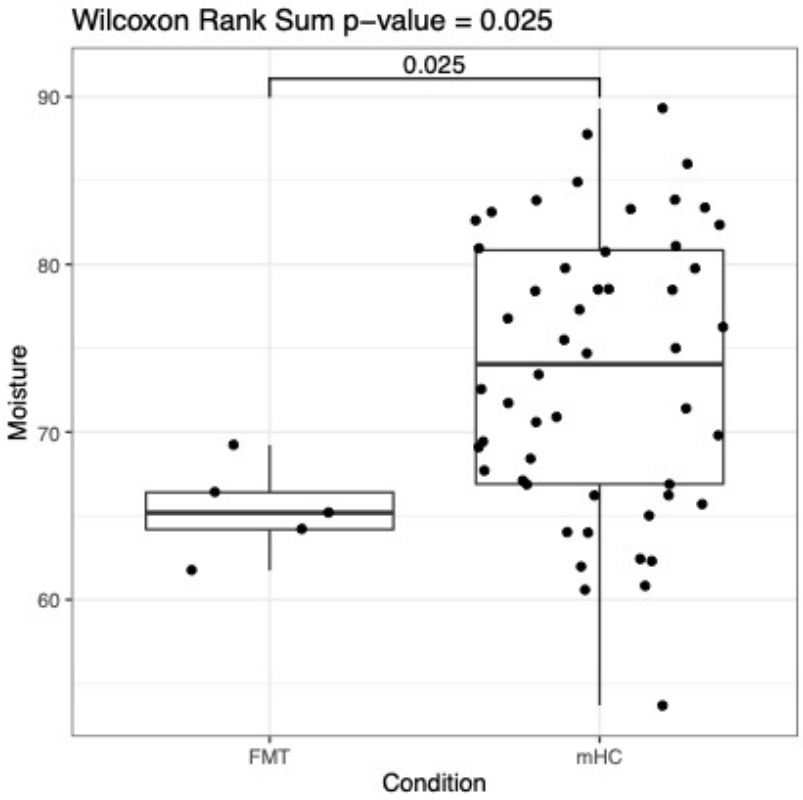
Supplementary Figure 4. Microbiota communities of the 2 patients on A) genus level and B) phylum level.



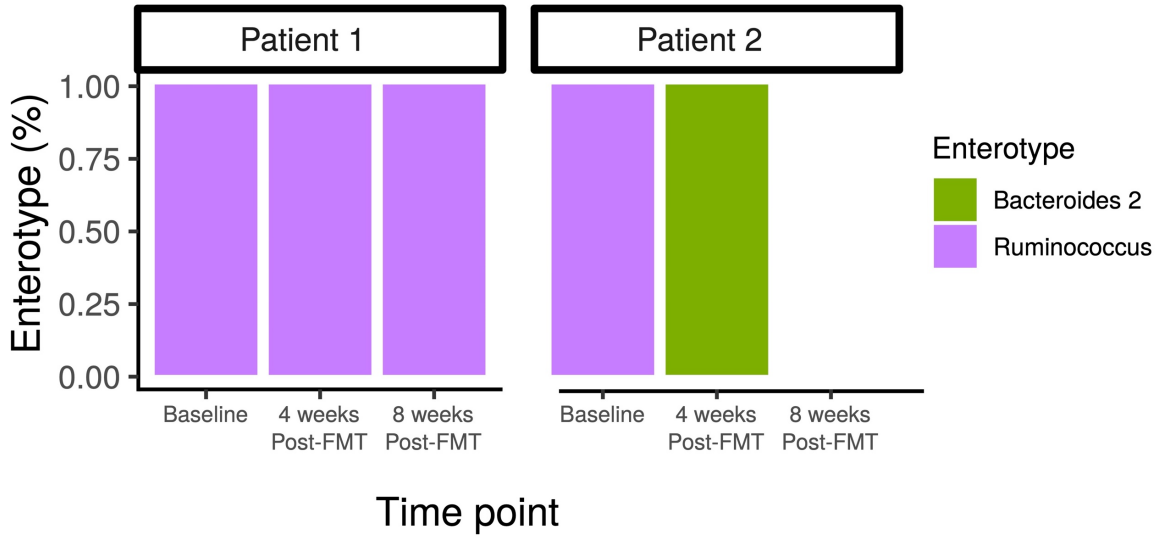
Supplementary Figure 5. A) Hierarchical clustering patients and time points using the ASV-QMP matrix based on Bray-Curtis dissimilarity and the ward.D clustering criteria. The clustering considered all the ASV, but only those significant according to the DESeq2 method (adjusted p-value < 0.05 and |effect size| > 2) were represented in the heatmap and marked with an asterisk. B) Bray-Curtis principal coordinates analysis. The arrows represent the envfit metadata covariation with the two principal components.



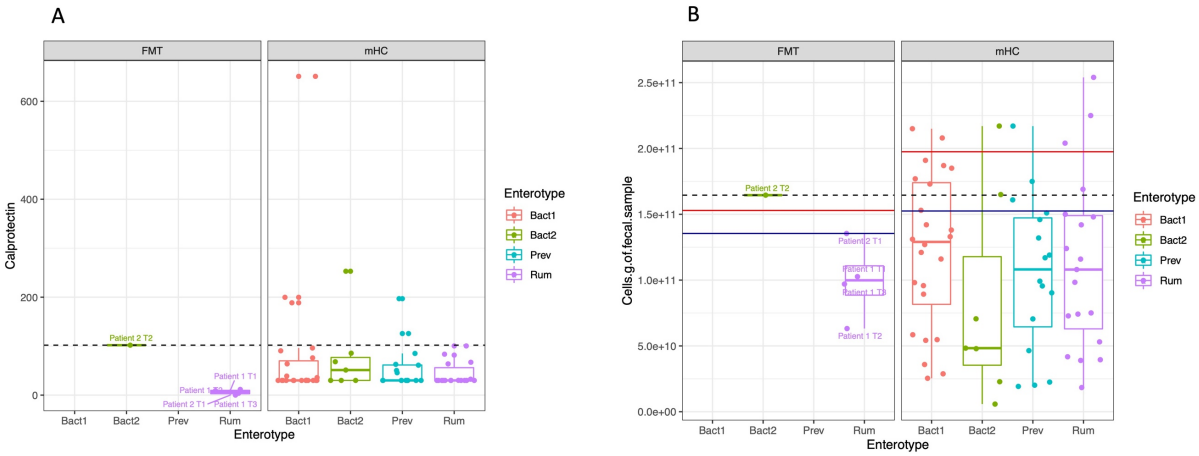
Supplementary Figure 6. Effect of the FMT over the transit time and local inflammation. Mixed-effects models (MEM) of the (A) moisture, (B) calprotectin and C) the bacterial load for both patients. Both measurements were modelled into a discrete manner and represented the results into the boxplot figures and in a continuous way, representing the MEM slope into the line plots. The grey area into the continuous MEM represents the 95% confidence level. The red, blue, and dark green colors represent the different time points. Patients are represented in orange and purple.



Supplementary Figure 7. Moisture levels of the 2 patients compared to a healthy population.



Supplementary Figure 8. Enterotypes of the 2 patients over the available time points.



Supplementary Figure 9. A) Fecal calprotectin levels in our FMT cases compared healthy subjects. B) Cell counts in our FMT cases versus healthy subjects.

Supplementary Table 1. Medication of patient 1									
Active Ingredient	Brand Name	Type	Time Period						
			28.11.18-12.12.18	13.12.18-18.12.18	19.12.18-20.12.18	21.12.18-26.12.18	27.12.18-08.01.19	09.01.19-11.01.19	12.01.19-31.01.19
Lamotrigin 250mg	Lamictal	Anticonvulsants	1	1	1	1	1	1	Discharged
Trazodon 100mg	Trittico	Antidepressant (SSRI)	2.5	2.5	2.5	2.5	2.5	2.5	Discharged
Macrogol 13.125g, Natrium chloride 350.7mg, Natriumhydrogencarbonate 178.5mg, Potassium chloride 46.6mg	Movicol	Laxative	3	0	0	0	0	0	Discharged
Psyllium 491.5mg	Metamucil	Laxative	3	0	0	0	0	0	Discharged
Bupropion 150mg	Wellbutrin	Antidepressant (NDRI)	0	1	0	0	0	0	Discharged
Vortioxetin 5mg	Brintellix	Antidepressant (serotonin modulator and stimulator)	0	0	0	1	2	3	Discharged

Note. Medication for TAU for depression and constipation for patient 1 during eight-weeks post-FMT. The time period is based on changes in medication prescription. The patient was discharged mid-January and medication intake was therefore not traceable. Provisional medication was available, yet not taken during the eight weeks.

Supplementary Table 2. Medication of patient 2									
Active Ingredient	Brand Name	Type	Time Period						
			17.02.20-20.02.20	21.02.20-26.02.20	27.02.20-04.03.20	05.03.20-10.03.20	11.03.20-12.02.20	13.03.20-19.03.20	20.03.20-23.03.20	24.03.20-14.04.20
Lamotrigin 100mg	Lamictal	Anticonvulsants	6	6	6	6	6	6	6	6
Trazodone hydrochloride 100 mg	Trittico	Antidepressant	1	1	1	1	1	1	1	0
Escitalopram 10mg	Escitalopram	Antidepressant	2	2	2	2	2	2	2	2
Lorazepam 1mg	Temesta	Benzodiazepine	2	1.5	1.5	1.5	1.5	1.5	1	1
Pregabalin 150mg	Pregabalin	Anticonvulsants	1	1	1	1	1	1.5	2	2
Chlorprothixen hydrochloride 50mg	Truxal	Antipsychotic	5	5	5	5	5	5	5	5
Clotiapin 40 mg	Entumin	Antipsychotic	0	0	0	1	1.5	0	0	0

Note. Medication for TAU for depression for patient 2 during eight-weeks post-FMT. The time period is based on changes in medication prescription. The provisional medication was available and taken as follows: 30 times Chlorprothixen hydrochloride 50mg, 4 times Chlorprothixen hydrochloride 15mg, 4 times Lorazepam 1mg (Temesta Expidet), 3 times Lorazepam 1mg (Temesta), 1 time Paraffin 1.9g (Paragol; laxative) and 2 times Natrium picosulfat-1-water 7.5mg (Laxoberon; laxative). Additionally, she has received intravenous Ketamine therapy 1/month as pain treatment since 2016.

Supplementary Table 3. Multivariate analysis of variance results table				
Variable	Fmodel	R2	p-value	BH adj p-value
Subject ID	2.577	0.462	0.100	0.267
BMI	2.577	0.462	0.100	0.267
Age	2.577	0.462	0.100	0.267
Calprotectin corrected	1.738	0.367	0.175	0.350
Time point	0.553	0.356	0.933	0.975
Moisture	0.776	0.205	0.733	0.975
HAMD	0.735	0.197	0.742	0.975
GSRS	0.619	0.171	0.975	0.975

Note. Statistics of the patient metadata according to adonis multivariate analysis of variance.

Supplementary Table 4. Medication and side effects on transit time and calprotectin					
Active Ingredient	Brand Name	Type	Effect on transit time	Effect on Calprotectin	References
Lamotrigin 100mg	Lamictal	Anticonvulsants	Diarrhea (Rare)	None	https://www.drugs.com/lamictal.html; https://www.fda.gov/media/79324/download
Trazodone hydrochloride 100 mg	Trittico	Antidepressant	diarrhea, constipation (Less common)	None	https://www.drugs.com/trazodone.html; https://www.mayoclinic.org/drugs-supplements/trazodone-oral-route/side-effects/drg-20061280
Escitalopram 10mg	Escitalopram	Antidepressant	Diarrhea (common*)		https://www.mayoclinic.org/drugs-supplements/escitalopram-oral-route/side-effects/drg-20063707
Lorazepam 1mg	Temesta	Benzodiazepine	constipation (incidence not known*)	None	https://www.mayoclinic.org/drugs-supplements/lorazepam-oral-route/side-effects/drg-20072296

Continued from Supplementary Table 4. Medication and side effects on transit time and calprotectin					
Pregabalin 150mg	Pregabalin	Anticonvulsants	diarrhea (rare); severe constipation (Incidence not known)	None	https://www.mayoclinic.org/drugs-supplements/pregabalin-oral-route/side-effects/drg-20067411
Chlorprothixen hydrochloride 50mg	Truxal	Antipsychotic	None	None	https://www.wikidoc.org/index.php/Chlorprothixene
Clotiapin 40 mg	Entumin	Antipsychotic	constipation (Incidence not known)	None	https://www.tabletwise.net/medicine/clotiapine
Bupropion 150mg	Wellbutrin	Antidepressant (NDRI)	Constipation	NA	https://compendium.ch/product/1075670-wellbutrin-xr-ret-tabl-150-mg
Vortioxetin 5mg	Brintellix	Antidepressant (serotonin modulator and stimulator)	Constipation, diarrhea	NA	https://compendium.ch/product/1323039-brintellix-filmtabl-5-mg

Continued from Supplementary Table 4. Medication and side effects on transit time and calprotectin					
Macrogol 13.125g, Natrium chloride 350.7mg, Natriumhydrogencarbonate 178.5mg, Potassium chloride 46.6mg	Movicol	Laxative	Increase of transit time	NA	https://compendium.ch/product/1080123-movicol-aromafrei-plv/mpro
Psyllium 491.5mg	Metamucil	Laxative	Increase of transit time	NA	https://compendium.ch/product/1098398-metamucil-n-mite-plv-5-8-g-orange

Note. *These side effects may go away during treatment as the subject gets adapted to the medicine. NA: not available, to the best of our knowledge, we could not find information on the effect on calprotectin.

References

1. Zimmerman M, Martinez JH, Young D, Chelminski I, Dalrymple K. Severity classification on the Hamilton Depression Rating Scale. J Affect Disord. 2013;150(2):384-8.

2. Fischer M, Allegretti J, Smith M, Klank M, Mendiola G, Vo E. A multi-center, cluster randomized dose-finding study of fecal microbiota transplantation capsules for recurrent Clostridium difficile infection. United Eur Gastroenterol J. 2015;3:561-71.

3. Lee CH, Steiner T, Petrof EO, Smieja M, Roscoe D, Nematallah A, et al. Frozen vs Fresh Fecal Microbiota Transplantation and Clinical Resolution of Diarrhea in Patients With Recurrent Clostridium difficile Infection: A Randomized Clinical Trial. JAMA. 2016;315(2):142-9.

4. Kao D, Roach B, Silva M, Beck P, Rioux K, Kaplan GG, et al. Effect of Oral Capsule- vs Colonoscopy-Delivered Fecal Microbiota Transplantation on Recurrent Clostridium difficile Infection: A Randomized Clinical Trial. JAMA. 2017;318(20):1985-93.

5. Falony G, Joossens M, Vieira-Silva S, Wang J, Darzi Y, Faust K, et al. Population-level analysis of gut microbiome variation. Science. 2016;352(6285):560-4.

6. Vandeputte D, Kathagen G, D'hoe K, Vieira-Silva S, Valles-Colomer M, Sabino J, et al. Quantitative microbiome profiling links gut community variation to microbial load. Nature. 2017;551(7681):507-11.

7. Prest EI, Hammes F, Kötzsch S, van Loosdrecht MC, Vrouwenvelder JS. Monitoring microbiological changes in drinking water systems using a fast and reproducible flow cytometric method. Water Res. 2013;47(19):7131-42.

8. Kirsch I, Deacon BJ, Huedo-Medina TB, Scoboria A, Moore TJ, Johnson BT. Initial severity and antidepressant benefits: a meta-analysis of data submitted to the Food and Drug Administration. PLoS Med. 2008;5(2):e45.

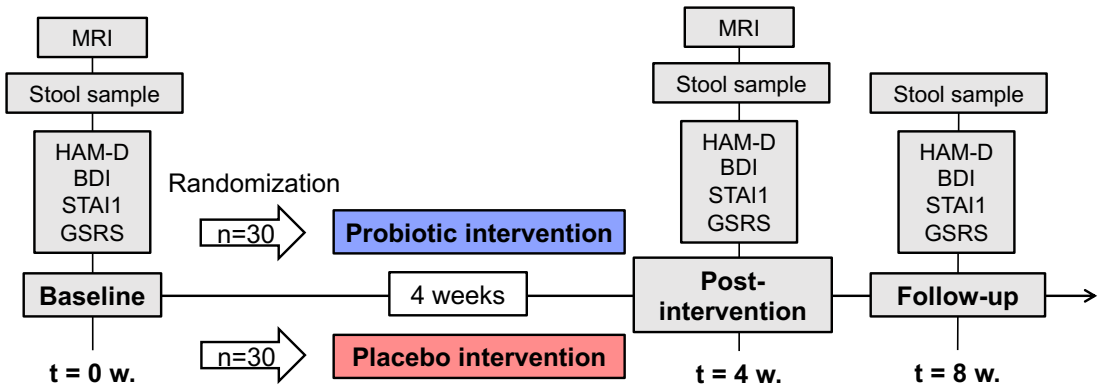
9. Vieira-Silva S, Sabino J, Valles-Colomer M, Falony G, Kathagen G, Caenepeel C, et al. Quantitative microbiome profiling disentangles inflammation- and bile duct obstruction-associated microbiota alterations across PSC/IBD diagnoses. Nat Microbiol. 2019;4(11):1826-31.

7.2.Supplementary Information study B)

Clinical, gut microbial and neural effects of a probiotic add-on therapy in depressed patients: A randomized-controlled trial

Supplementary information (SI)

Supplementary Methods



Supplementary Figure 1. Study design. MRI = magnetic resonance imaging; HAM-D = Hamilton Rating Scale for Depression; BDI = Beck Depression Inventory; GSRS = Gastrointestinal Symptom Rating Scale; STAI1 = State-Trait Anxiety Inventory 1.

Medication

To monitor treatment as usual, medication intake was extracted for each patient from the hospital information system. Antidepressants and antipsychotics were transformed into dose equivalents based on the defined daily dose (DDD) method, which is described as the "*assumed average maintenance dose per day for a drug used for its main indication in adults»*.[1] For each patient, detailed information is depicted in Table S1. Different antidepressants and antipsychotics were summarized using the DDD. Furthermore, the

specific period of intake was considered as the drugs were not necessarily administered over the whole study period.

Supplementary Table 1. Antidepressant and antipsychotic medication per patient in mean defined daily dose (DDD) over the four-week intervention period. Only patients that finished the intervention are included.

Group	Patient ID	Name	DDD
Probiotics	Patient 1	mirtazapine, fluoxetine, trimipramine, lithium, olanzapine, chlorprothixene, quetiapine	3.45
	Patient 2	venlafaxine, lithium, quetiapine	0.76
	Patient 3	venlafaxine, quetiapine	2.33
	Patient 4	lithium, quetiapine	0.75
	Patient 5	bupropion, trazodone	1.47
	Patient 6	venlafaxine, trazodone, quetiapine	2.47
	Patient 7	quetiapine	0.01
	Patient 8	sertraline, quetiapine	3.22
	Patient 9	escitalopram	2.00
	Patient 10	venlafaxine, quetiapine	4.02
	Patient 11	duloxetine, quetiapine	1.12
	Patient 12	lithium, citalopram, quetiapine, olanzapine	4.33
	Patient 13	duloxetine, quetiapine	1.04
	Patient 14	duloxetine	0.50
	Patient 15	mirtazapine, sertraline, quetiapine	5.26
	Patient 16	venlafaxine, quetiapine	3.10
	Patient 17	sertraline, mirtazapine	3.25
	Patient 18	bupropion, trimipramine, clotiapine, pipamperone	0.93
	Patient 19	duloxetine	0.50
	Patient 20	bupropion, quetiapine	1.13
	Patient 21	duloxetine	1.00

Placebo	Patient 1	vortioxetine, quetiapine	1.57
	Patient 2	sertraline, vortioxetine, clotiapine	1.91
	Patient 3	duloxetine, olanzapine	3.00
	Patient 4	venlafaxine, olanzapine, clotiapine	3.02
	Patient 5	duloxetine, risperidone, quetiapine	1.03
	Patient 6	agomelatine	1.74
	Patient 7	venlafaxine	2.73
	Patient 8	mirtazapine, quetiapine	1.06
	Patient 9	bupropion	1.00
	Patient 10	bupropion	0.45
	Patient 11	vortioxetine	1.00
	Patient 12	fluoxetine, olanzapine	1.45
	Patient 13	escitalopram, olanzapine	2.97
	Patient 14	lithium, olanzapine, pipamperone	0.77
	Patient 15	venlafaxine, vortioxetine, olanzapine	3.99
	Patient 16	duloxetine	1.55
	Patient 17	mirtazapine	1.50
	Patient 18	trazodone, bupropion	0.60
	Patient 19	trazodone, bupropion, duloxetine	1.30
	Patient 20	escitalopram	2.00
	Patient 21	escitalopram, lithium	4.00
	Patient 22	venlafaxine, mirtazapine, bupropion, clotiapine	2.61
	Patient 23	vortioxetine, quetiapine, olanzapine, chlorprothixene	1.54
	Patient 24	fluoxetine, agomelatine, pipamperone, quetiapine	5.26
	Patient 25	vortioxetine	2.07
	Patient 26	vortioxetine, clotiapine, quetiapine	2.27

Notes. DDD = defined daily dose.

Study procedure

Patients had the right to withdraw from the study without being obliged to give reason. Adverse events challenging the health of the patients, severe protocol violations or administrative troubles resulted in withdrawal in the best interest of the patient. Block randomization was performed in a 1:1 ratio by an independent researcher using a computer-based randomization algorithm to avoid systematic biases. Therefore, investigators and assessors were blinded during data collection and analysis. Patients were informed about the allocation after the follow-up assessment by an independent researcher.

Statistical analysis of clinical measures

In a first step, analyses were run on an intention-to-treat (ITT) basis with all participants that completed the post-intervention assessment. In a modified ITT analysis (mITT),[2] only patients with compliance >65% were included. This cut-off was based on evidence showing that patients receiving antidepressant medication took in average 65% of the prescribed amount.[3] Single missing values in questionnaires were imputed with the k-nearest-neighbor method.[4] HAM-D and BDI total scores over all time points were sqrt-transformed since distributions were non-normal. GSRS scores were log-transformed due to a strong negative skew. All statistical analyses on behavioral data were performed using R Version 3.6.3 including packages such as 'lme4'.[5] Treatment response was dichotomized according to the Clinical Global Impression (CGI) criteria as a change >57% in HAM-D scores compared to baseline measures.[6]

Gut microbiome

Bacterial DNA extraction and sequencing

The fecal DNA was extracted following the protocol described in Falony et al..[7] Summarily, DNA was extracted from 150-200mg of the frozen samples using MagAttract PowerMicrobiome DNA/RNA KF kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. The V4 region of 16S rRNA genes was amplified using the 515F /806R primer

pair and purified using the QIAquick PCR Purification Kit. Sequencing was performed using the Illumina MiSeq platform (MiSeq Reagent Kit v2, Illumina, San Diego, USA).

16s rRNA data processing

Amplicon data from the 16S rRNA gene was analyzed following the DADA2 pipeline specifications.[8] Briefly, the first 30bp were removed, and the sequence length was set to 130bp and 200bp for the forward and reverse strands, respectively. The sequence error rate, dereplications, the inferred composition of the sample, and the chimera removal were done using the DADA2 default parameters. The taxonomic assignation was done using the DADA2 RDP implementation (R packages “dada2” function “assignTaxonomy”) with the rdp_train_set_16 as reference, similarly the amplicon sequence variant (ASV) annotation was done using the GTDB_bac120_arc122_ssu_r202_Species trainset (R packages “dada2” function “addSpecies”). The relative abundance was presented at the ASV level and summarized to the genus level.

Microbial load measurement

The microbial load of the study cohort was measured by flow cytometry as described previously.[9] Briefly, 200-250 mg frozen (-80°C) fecal aliquots were diluted in saline solution (0.85% NaCl; VWR International, Germany) and filtered using a sterile syringe filter (pore size of 5 µm; Sartorius Stedim Biotech GmbH, Göttingen, Germany). Next, 1 mL of the microbial cell suspension obtained was stained with 1 µL SYBR Green I (1:100 dilution in DMSO; Thermo Fisher Scientific, Massachusetts, USA) and incubated for 15 min in the dark at 37°C. The flow cytometry analysis was performed using a C6 Accuri flow cytometer (BD Biosciences, New Jersey, USA) based on Prest et al..[10] Fluorescence events were monitored using the FL1 533/30 nm and FL3 >670 nm optical detectors. The BD Accuri CFlow software was used to gate and separate the microbial fluorescence events on the FL1/FL3 density plot from the fecal sample background. A threshold value of 2000 was applied on the FL1

channel. Based on the exact weight of the aliquots analyzed, cell counts were converted to microbial loads per gram of fecal material.

Quantitative microbiome profiling

The quantitative microbiome profiling (QMP) matrix was built as described by Vandeputte and colleagues.[9] In brief, samples were downsized to even sampling depth, defined as the ratio between sampling size (16S rRNA gene copy number-corrected sequencing depth) and microbial load (the average total cell count per gram of frozen fecal material). 16S rRNA gene copy numbers were retrieved from the rRNA operon copy number database rrnDB33. The final matrices were represented as the "QMP" matrix and the "even sample depth rarefied matrix", which is the number of reads per sample rarefied according to the sampling depths determined by the sample's cell counts.

Fecal moisture content

The fecal moister content was determined as the percentage of mass loss after lyophilization from 0.2g frozen aliquots of non-homogenized fecal material as previously done.[7]

Fecal calprotectin measurement

Fecal calprotectin concentrations were determined using the fCAL ELISA Kit (Bühlmann, Amherst, USA). The measurements were done on frozen fecal material (-80°C).

Enterotyping

The 16s rRNA bacterial profiles were collapsed at the genus level and integrated along with the FGFP cohort as done in the previous work.[7] The identification of the enterotypes was accomplished with the Dirichlet-multinomial Model (DMM) approach in R (library "DirichletMultinomial" function "dmn"). To compare enterotype distributions between depressed patients and healthy subjects, a group of 93 healthy subjects that was matched by

age, BMI and sex with the study sample (sex $\chi 2(1)=0$, p=1; BMI W=5225, p=.96; age W=5168.5, p=0.94) was taken from the Belgian Flemish Gut Flora (FGFP) cohort.[11]

Diversity analysis

Diversity analysis was performed using the R statistical software (v3.6.3). The beta diversity analysis from the 16S rDNA amplicon sequence variant (ASV) data was estimated. The Bray-Curtis index (library "Vegan", function "vegdist") was used to estimate the dissimilarities between samples in the QMP even sampling depth ASV table. Low frequent ASV data (80% of zero data) were removed before the dissimilarity estimation. A distance-based redundancy analysis (dbRDA) (library "Vegan" function "capscale") was performed to reduce dimensionality in the taxonomic and functional distance matrix. The Permutational Multivariate Analysis of Variance Using Distance Matrices (ADONIS test) (library "vegan" function "adonis") clinical and metadata variables. Clinical measures were correlated into the ordination using the function “envfit” (library "vegan"). The adonis and envfit p-values were adjusted using the Benjamini-Hochberg method (library "stats" function "p.adjust"). Observed richness, Shannon and Inverse Simpson index (library "phyloseq" function "estimate_richness") and Pielou's evenness (library "microbiome" function "evenness") indices were estimated at the genus level for each sample.

Statistical analysis of gut microbiota data

Differences in the frequency of enterotypes was determined through Chi-squared tests (library "stats" function "chisq.test") and the difference in the frequency of the enterotypes over time within the placebo and probiotic group by symmetry tests for paired contingency tables (library "rcompanion" function "nominalSymmetryTest"); p-values were adjusted using Benjamini-Hochberg's correction (library "stats" function "p.adjust"). The effect of the intervention on enterotypes was examined using a binomial generalized linear model (library "lme4" function "lmer"). The dependent variable was the enterotype two-level categorization (enterotype and non-enterotype) for all four different enterotypes. The fixed effect was the

time*group interaction, moisture, calprotectin, sex, body-mass index (BMI), and age; and the subject ID was set as random intercept. An ANOVA test and the AIC determined the significance and feature selection model (library "car" function "Anova"). The effect of probiotics in reducing Bacteroides 2 prevalence was estimated by means of an odds ratio (OR) between the frequency of the Bacteroides 2 enterotype of the placebo and probiotic group and the FGFP matched controls (library "epitools" function "oddsratio").

Associations between bacterial taxa (ASV and genus) and time were estimated using a zero-inflated mixed effect negative binomial model if the taxa prevalence were between 20 and 80%, and mixed effect negative binomial model, if the taxa prevalence were above 80%, as suggested by Zhang and Yi.[12] The model considers time as fixed effect; the subject ID was modeled using a random intercept (library "NBZIMM" function "mms" and library "glmmTMB" function "glmmTMB"). The zero-inflation parameter was determined using a single zero-inflation parameter applied to all observations (~1). An ANOVA test (library "car" function "Anova") determined the significance of the overall effect of time over the taxa; meanwhile, the Wald-test was used to determine the significance of the three levels of the time variable (library "base" function "summary"). All p-values were adjusted using Benjamini-Hochberg's correction (library "stats" function "p.adjust"), a taxon was considered to be associated with time if the ANOVA and all the Wald-test corrected p-values were significant and if the mean abundance of the post-intervention and follow-up were congruently higher or lower in comparison to the baseline. The taxa*time interactions were confounded by fecal moisture, sex, BMI, and age using the step function (library "stats" function "step"). The analyses were done independently for the placebo and probiotic group. The taxonomic time*group comparison was modelled using the before mentioned strategy but setting the time*group interaction as the fixed effect and the subject ID as random intercept (library "glmmTMB" function "glmmTMB").

A similar approach was made to estimate the associations between bacterial taxa and behavioral measures and fecal calprotectin. Taxa were modelled as a mixed effect negative

binomial or as a mixed effect zero-inflated negative binomial model depending on its zero abundance as described above. The model considers clinical measures and fecal calprotectin as fixed effects; the subject ID as random effects (library "glmmTMB" function "glmmTMB"). Independent models were done for the HAM-D, GSRS, BDI, STAI1 and fecal calprotectin. An ANOVA test (library "car" function "Anova") determined the significance of taxa interactions. All p-values were adjusted using Benjamini-Hochberg's correction (library "stats" function "p.adjust"). The taxon-variable associations were confounded by fecal moisture, sex, BMI, and age using the step function (library "stats" function "step"). Analyses were done separately per group. All statistical analyses were done using R (v3.6.3).

Visualization of gut microbiota analyses

The taxonomic summarization at the Phylum level was visualized into a barplot. All visualizations such as these barplots, boxplots and PcoA were done using the ggplot package in R.

Brain structure and function

Participants for imaging

For the analyses of the imaging data, data of 32 patients were available after excluding non-compliant patients ($N_{probiotics}$=14, $N_{placebo}$=18). Furthermore, an additional sample of 20 healthy controls (age: 40.25 ±10.91 years; 4 women) was used to be able to examine which structures are generally affected in the depressive patients compared to healthy people.

Image acquisition and data analysis

Anatomical and functional image acquisition was carried out on a 3T Siemens Magnetom Prisma whole-body scanner (Siemens Medical Solutions, Erlangen, Germany) with a 20-channel head coil. For structural data, an anatomical T1-weighted image acquisition followed a three-dimension (3D) magnetization-prepared rapid gradient-echo (MP-RAGE) sequence

pulse[13] with a spatial resolution of 1×1×1 mm^3 (slice thickness: 1.0 mm, 176 sagittal slices; time of repetition (TR) = 2000ms; echo time (TE)=3.37ms; flip angle (FA)=8°; field of view (FOV)=256×256mm^2). To reveal structural brain changes due to the probiotic intervention, a voxel based morphometry was performed using the Computational Anatomy Toolbox (CAT) toolbox.[14] A flexible factorial design with the factors subject ID, group and time was used for data analysis.

For functional data, functional T2*-weighted images were acquired using a blood-oxygen-level-dependent (BOLD) sensitive, interleaved diffusion-weighted echo planar imaging (EPI) sequence with a spatial resolution of 3×3×3mm^3 (slice thickness: 3.0mm, 39 transversal slices; TR=2500ms; TE=30ms; FA=82°; FOV=228×228mm^2). The task included 10 different facial identities, each presented twice in the categories neutral, 50% and 100% intensity of fear; resulting in 60 faces in total. Faces were presented for 2 sec in a pseudo-random order avoiding successive presentations of the same face. Between the faces, a fixation cross was presented for a duration of 2-8 sec. As task, patients had to indicate the face's gender.

Data analysis was performed with SPM12 (http://www.fil.ion.ucl.ac.uk/spm/). All volumes were realigned to the first volume, coregistered to the anatomical volume, normalized to the MNI305 T1 template and finally smoothed with a 6 mm (FWHM) isotropic Gaussian kernel. During model specification, onset times for each trial of neutral, 50%, and 100% fearful faces (event-related design) and the duration of the fixation cross (block design) were convolved with a canonical hemodynamic response (HPR) function. Serial correlations were removed with a first-order autoregressive model, and a high-pass filter (128 sec) was applied to remove low-frequency noise. The six movement parameters were further included as nuisance covariates.

Each trial of the neutral, 50% and 100% fearful faces was subsequently contrasted against the fixation cross.[15] Afterwards, the activation changes over time were calculated for each face category by subtracting the activation contrasts in the post-intervention from the baseline. These subject-specific contrast images were propagated to the second-level analysis where a full factorial design with the two factors group (probiotic vs placebo) and faces (neutral, 50%

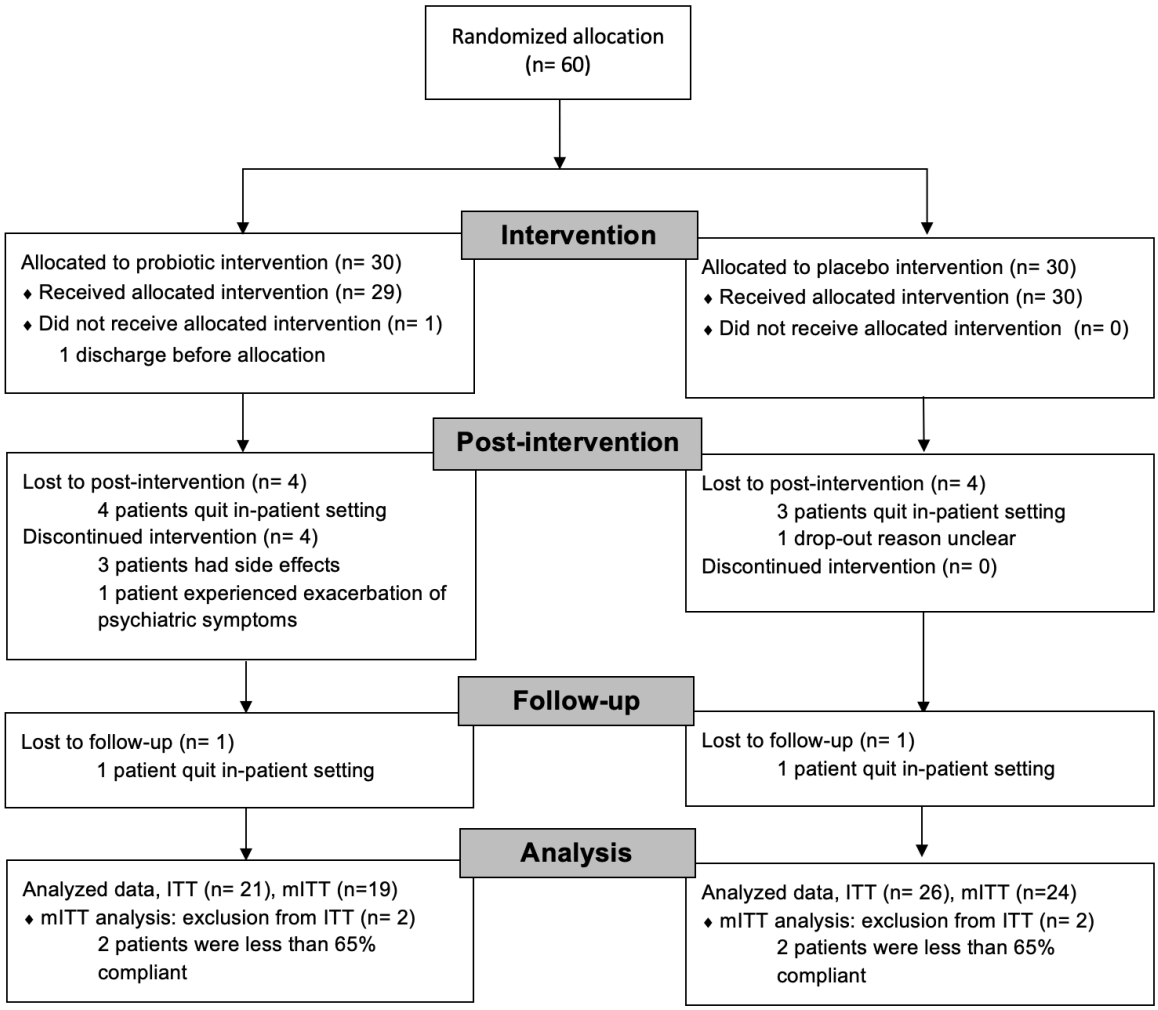
and 100% fearful) was used. Moreover, t-tests for neutral, semi-fearful and fearful faces were conducted to reveal activation changes between MDD patients and healthy controls during fearful face processing.

For imaging analyses regarding probiotics and placebo groups, we set the intensity threshold of the peak-voxel to a p-value of 0.001, uncorrected, and the minimal cluster size threshold k to 10 voxels. Additionally, we demanded a p-value of 0.05, familywise error correct, on cluster level to identify a cluster as significant. For the contrasts between patients and healthy controls we set the intensity threshold of the peak-voxel to a p-value of 0.05, familywise error corrected, and the minimal cluster size threshold k to 10 voxels.

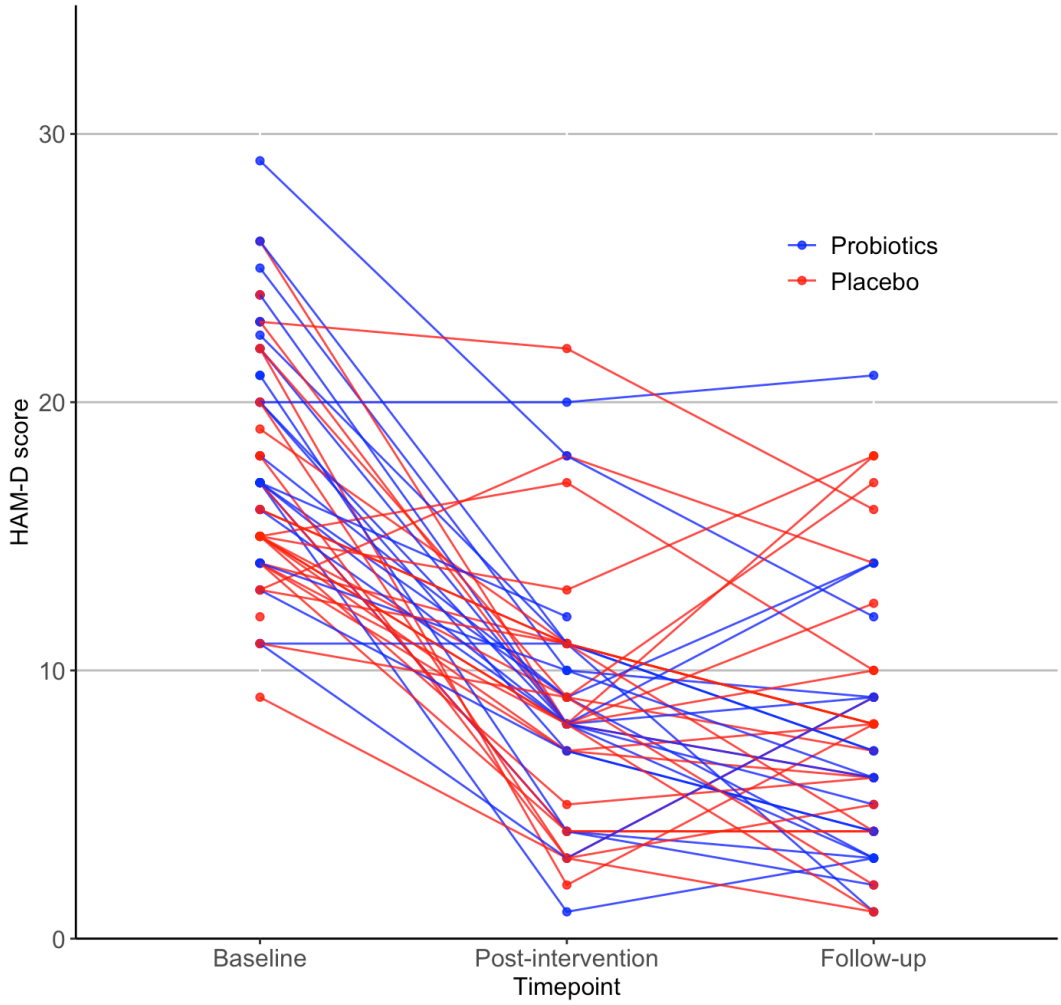
Supplementary results

Missing data analysis

The 12 patients who dropped out of the study during the intervention had equal HAM-D baseline scores than patients who completed the trial ($t(17.38)=-0.64$, $p=.53$). Furthermore, dropouts were not associated with gender ($\chi 2(1, 59)=0.06$, $p=.80$) and age ($t(17.38)=1.66$, $p=.12$).



Supplementary Figure 2. CONSORT diagram of participants of the probiotics study. ITT = intention-to-treat; mITT = modified intention-to-treat.



Supplementary Figure 3. Individual trajectories of depressive symptoms (HAM-D) in the intention-to-treat (ITT) sample. HAM-D = Hamilton Rating Scale for Depression.

Supplementary Table 2. Results of ANOVA over linear mixed models predicting depression scores (HAM-D) for ITT and mITT samples.

	HAM-D[a]							
	ITT sample				mITT sample			
Main effect	df	MS	F	*p*	df	MS	F	*p*
Group	1	0.00	0.001	.98	1	0.08	0.23	.64
Time	2	34.65	98.28	**<.001**	2	34.27	100.56	**<.001**
Time*group	2	0.79	2.23	.11	2	1.16	3.4	**.04**

Notes. [a] sqrt- transformed; HAM-D = Hamilton Rating Scale for Depression; ITT = intention-to-treat; mITT = modified intention-to-treat; *df* = degrees of freedom; MS = mean squares.

Supplementary Table 3. Results of ANOVA over linear mixed models predicting self-reported depressive symptoms (BDI) for ITT and mITT samples.

	BDI[a]							
	ITT sample				mITT sample			
Main effect	*df*	MS	*F*	*p*	*df*	MS	*F*	*p*
Group	1	0.071	0.12	.73	1	0.38	0.62	.43
Time	2	32.99	54.68	**<.001**	2	31.46	51.46	**<.001**
Time*group	2	0.267	0.44	.64	2	0.73	1.2	.31

Notes. [a] sqrt- transformed; BDI = Beck Depression Inventory; ITT = intention-to-treat; mITT = modified intention-to-treat; *df* = degrees of freedom; MS = mean squares.

Supplementary Table 4. Results of ANOVA over linear mixed models predicting self-reported anxiety (STAI1) for ITT and mITT samples.

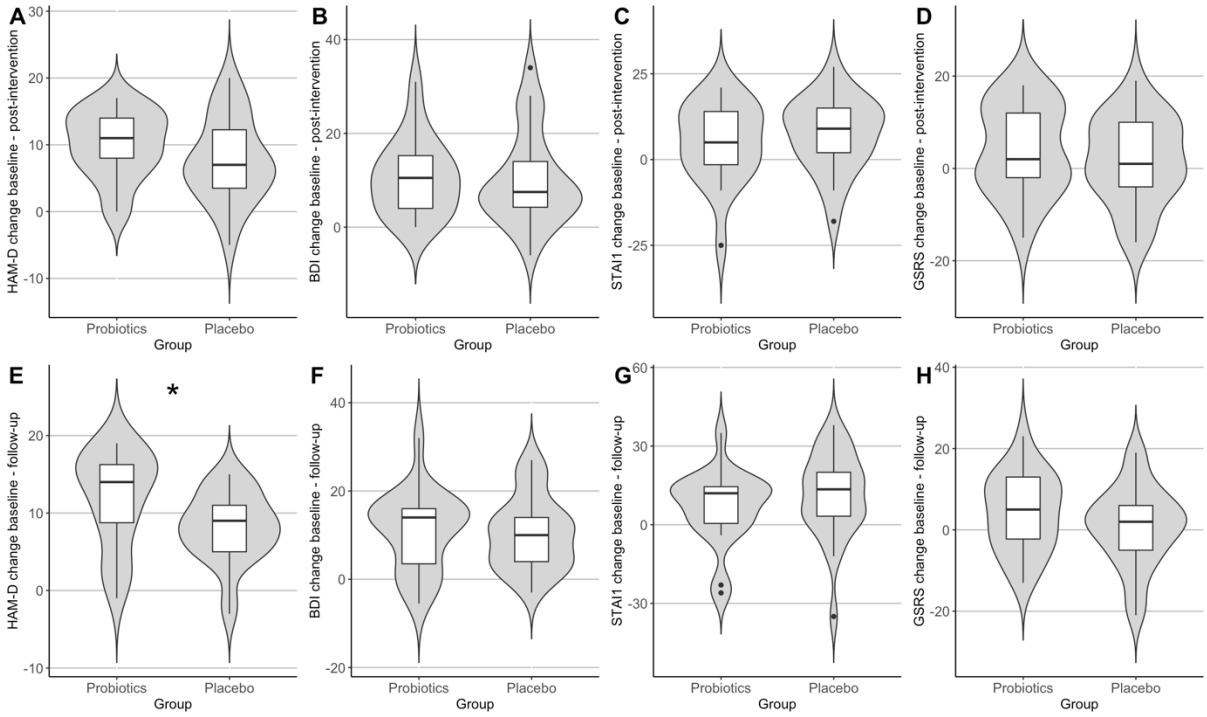
	STAI1							
	ITT sample				mITT sample			
Main effect	*df*	MS	*F*	*p*	*df*	MS	*F*	*p*
Group	1	4.19	0.05	.82	1	33.28	0.402	.53
Time	2	1005.9	12.56	**<.001**	2	824.79	9.983	**<.001**
Time*group	2	75.0	0.94	.40	2	38.25	0.463	.63

Notes. STAI1 = State-Trait Anxiety Inventory 1; ITT = intention-to-treat; mITT = modified intention-to-treat; *df* = degrees of freedom; MS = mean squares.

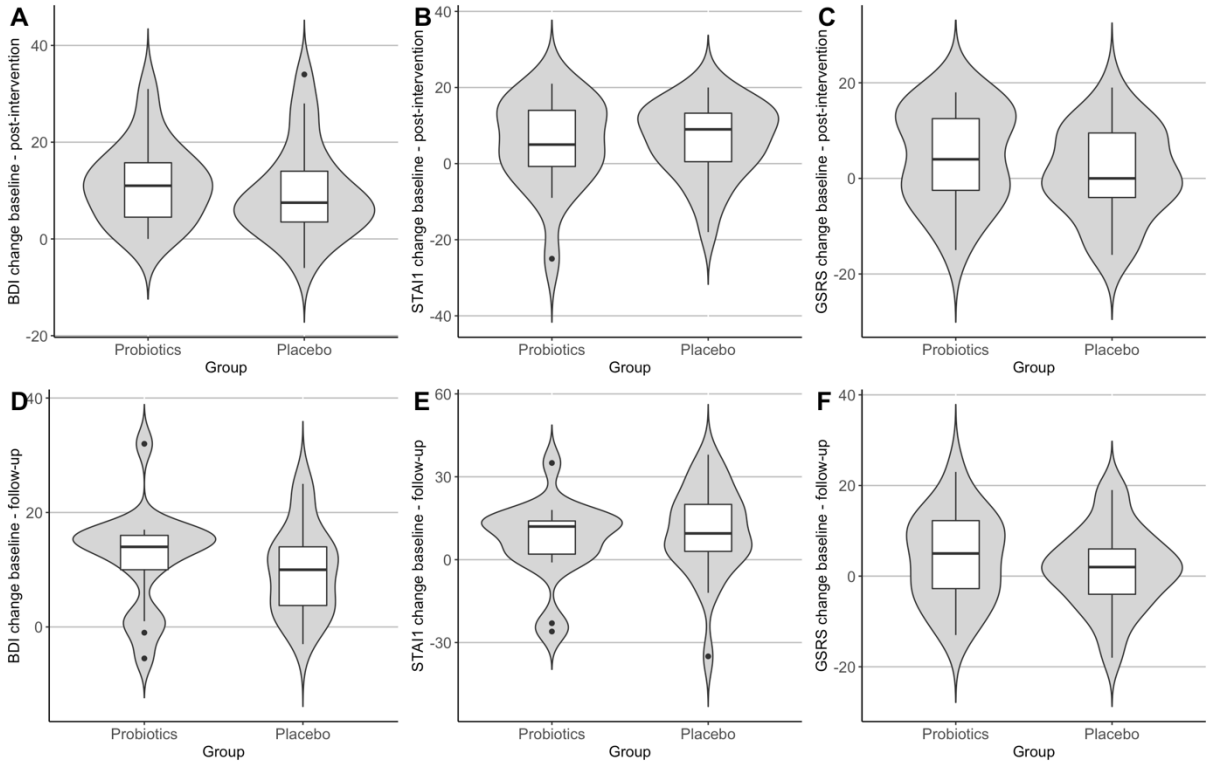
Supplementary Table 5. Results of ANOVA over linear mixed models predicting gastrointestinal symptoms (GSRS) for ITT and mITT samples.

	GSRS[a]							
	ITT sample				mITT sample			
Main effect	*df*	MS	*F*	*p*	*df*	MS	*F*	*p*
Group	1	0.13	0.34	.56	1	0.03	0.51	.48
Time	2	1.27	3.35	**<.05**	2	0.17	3.16	**<.05**
Time*group	2	0.42	1.11	.33	2	0.07	1.3	.28

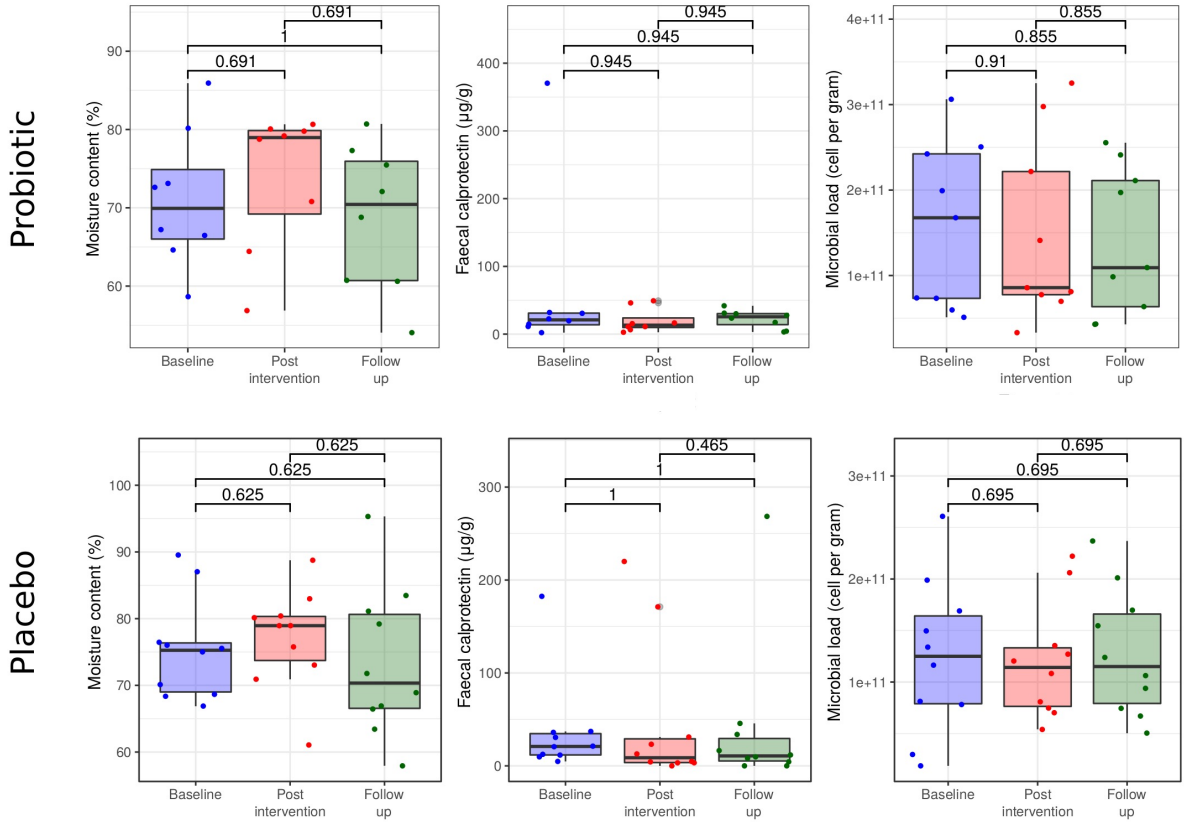
Notes. [a]log-transformed; GSRS = Gastrointestinal Symptom Rating Scale; ITT = intention-to-treat; mITT = modified intention-to-treat; *df* = degrees of freedom; MS = mean squares.



Supplementary Figure 4. Change scores from baseline to post-intervention (A, B, C, D) and from baseline to follow-up (E, F, G, H) of primary and secondary clinical outcomes in the intention-to-treat (ITT) sample. HAM-D = Hamilton Rating Scale for Depression; BDI = Beck Depression Inventory; STAI1 = State-Trait Anxiety Inventory 1; GSRS = Gastrointestinal Symptom Rating Scale.



Supplementary Figure 5. Change scores from baseline to post-intervention (A, B, C) and from baseline to follow-up (D, E, F) of secondary clinical outcomes in the modified intention-to-treat (mITT) sample. BDI = Beck Depression Inventory; STAI1 = State-Trait Anxiety Inventory 1; GSRS = Gastrointestinal Symptom Rating Scale.

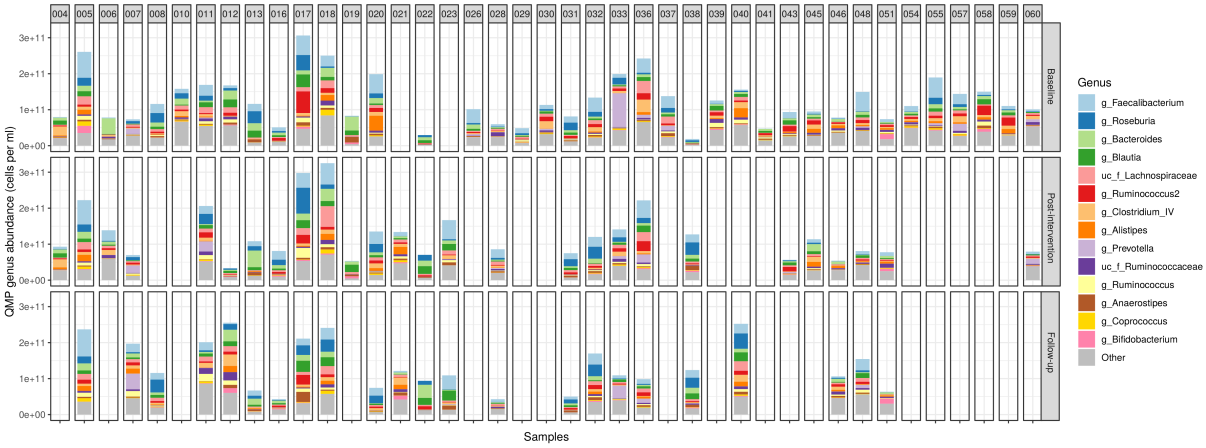


Supplementary Figure 6. Moisture, calprotectin and cell counts in fecal samples in both study groups and all time points.

Supplementary Table 6. Group comparisons of moisture, cell counts or calprotectin between both study groups at all time-points.

	Time point	Probiotics group	Placebo group	p	p-BH
Cells per gram of faeces, mean (SD)	Baseline	129849628868.111 (72011859905.177)	121182026443.551 (60252192580.798)	0.967	0.976
	Post-intervention	133487145556.333 (88477574418.402)	115298882513.253 (55271865891.772)	0.685	0.976
	Follow-up	138348498920.173 (81311664488.295)	134999480238.176 (64100242202.505)	0.976	0.976
Fecal calprotectin, µg/g, mean (SD)	Baseline	42.901 (81.021)	47.267 (75.391)	0.607	0.976
	Post-intervention	30.819 (24.032)	41.525 (70.191)	0.311	0.976
	Follow-Up	28.055 (18.453)	43.505/ (75.033)	0.717	0.976
Moisture, %, mean (SD)	Baseline	72.619 (6.396)	72.978 (8.483)	0.857	0.976
	Post-intervention	74.521 (8.115)	76.568 (6.796)	0.512	0.976
	Follow-up	67.505 (9.527)	74.322(10.495)	0.169	0.976

Notes. BH = Benjamini-Hochberg correction.

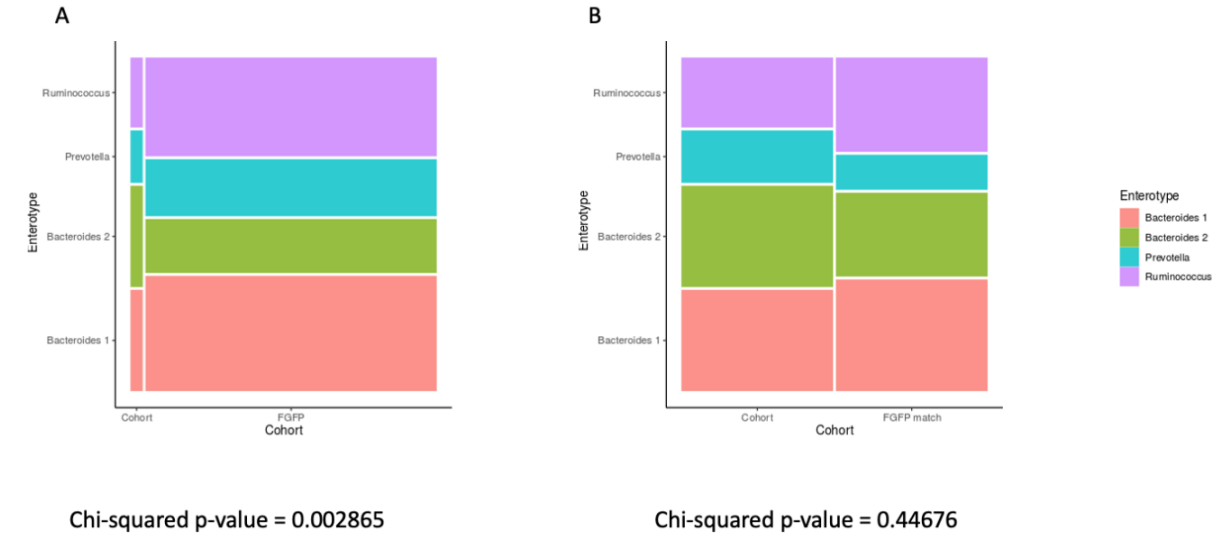


Supplementary Figure 7. Microbiome composition per subject and time point.

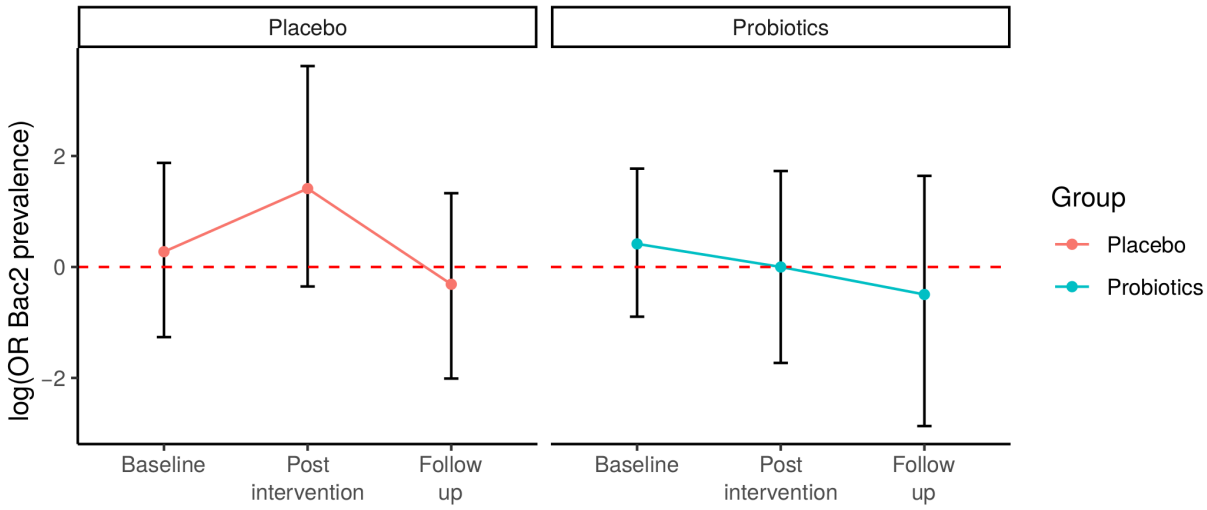
Enterotype distribution compared to healthy samples

The abundance of the Bacteroides 2 enterotype was higher in our study sample than in the FGFP sample (Supplementary Figure 8A) but when comparing the depressed patients with a matched subset of the FGFP, Bacteroides 2 prevalence was not significantly higher (χ^2, adj p>.1) (Supplementary Figure 8B).

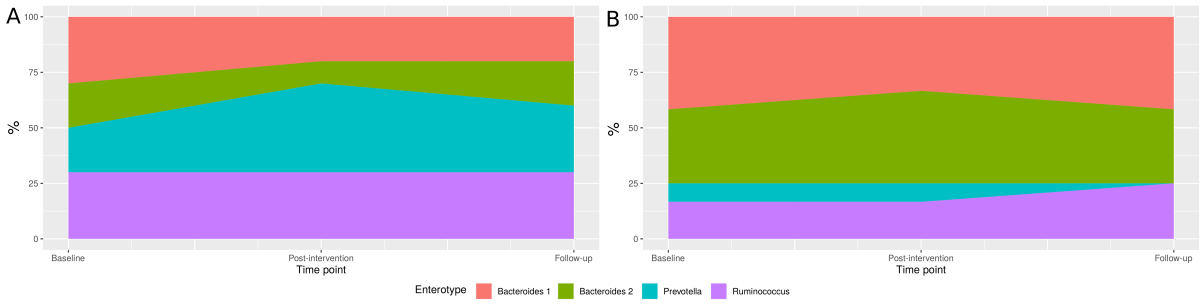
The OR between the study groups and the FGFP showed a non-significant decrease of the Bacteroides 2 enterotype in the probiotic group and contrary, the placebo showed a peak in the increase at the post-intervention (Supplementary Figure 9). However, the OR changed not significantly over time (OR χ^2 adjusted p>0.1).



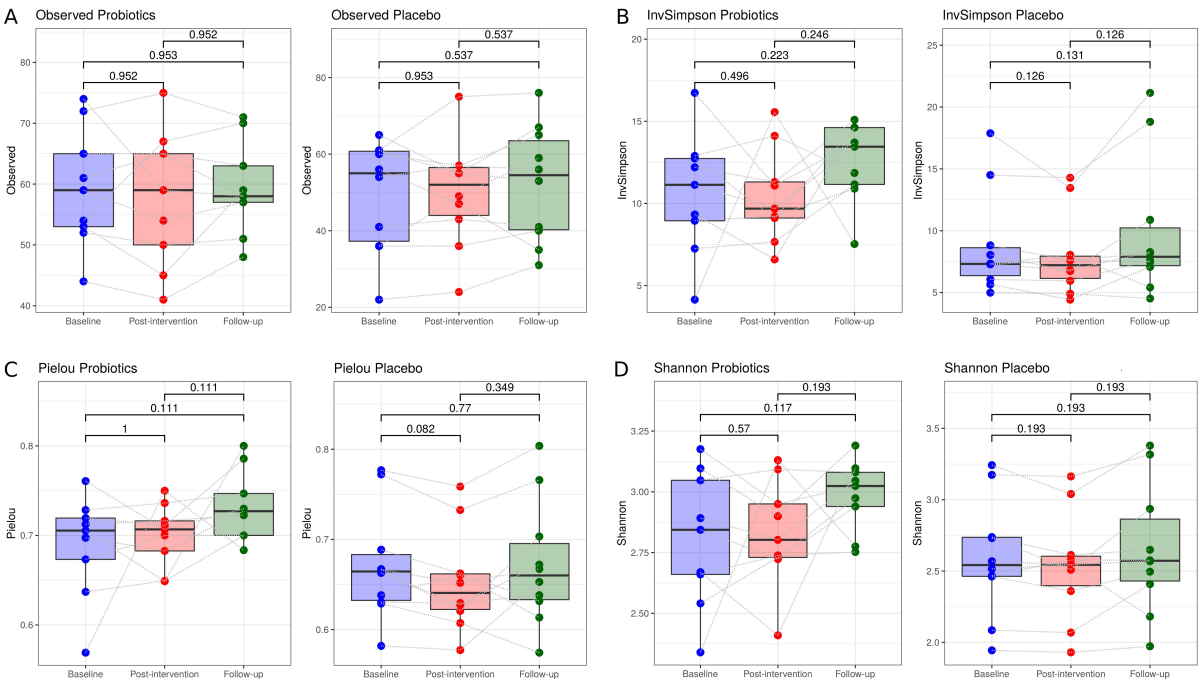
Supplementary Figure 8. Enterotype distribution of depressed subjects (combined probiotics and placebo group) compared to the healthy FGFP cohort (A) and the matched healthy sample (B).



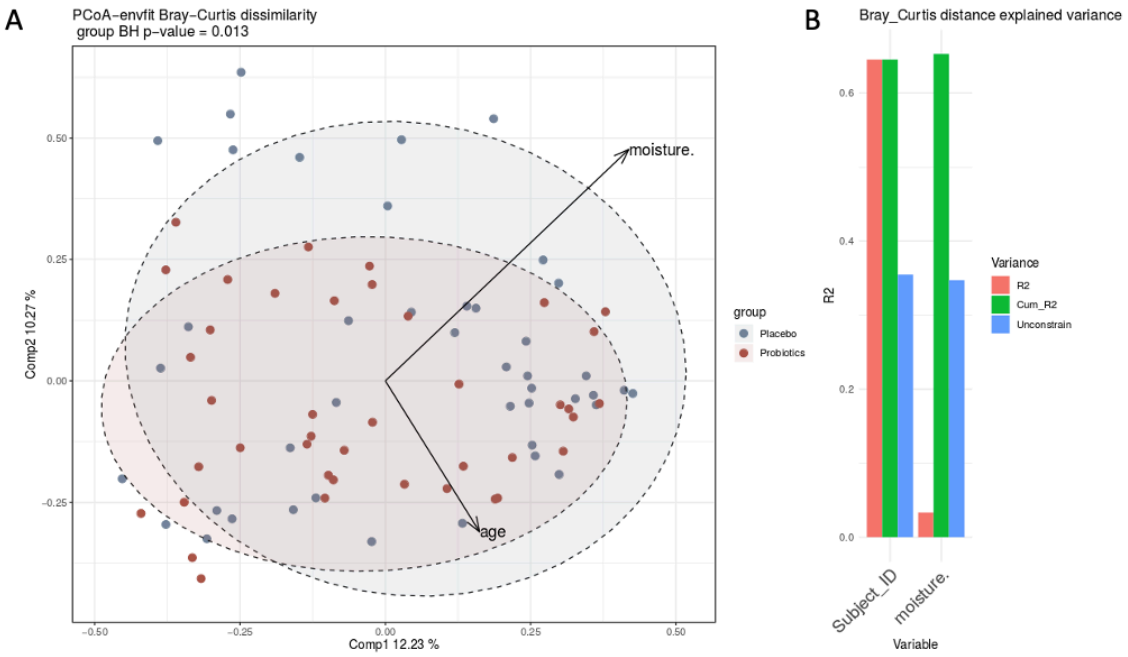
Supplementary Figure 9. ***Odds Ratio (OR) changes over time per study group. Y-axis is log-transformed***.



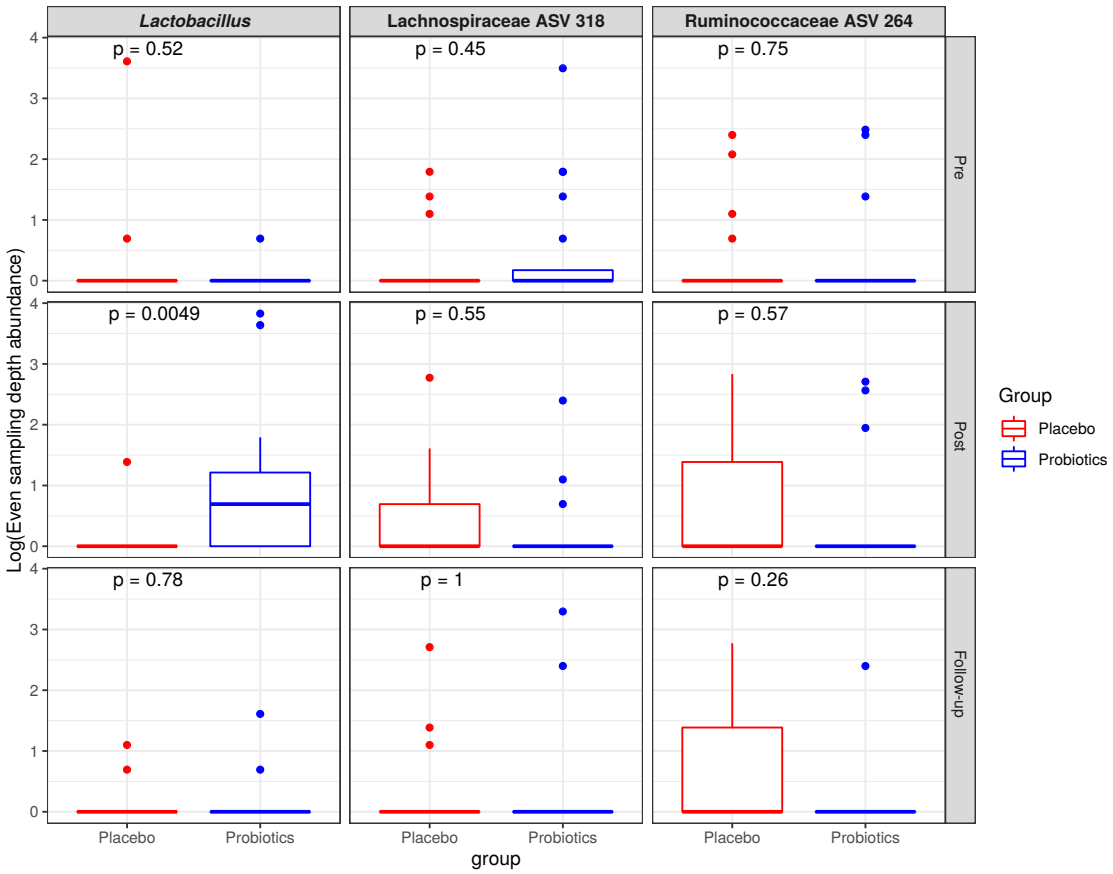
Supplementary Figure 10. Enterotype distribution over time in (A) the probiotics and (B) placebo group.



Supplementary Figure 11. Alpha-diversity indices such as (A) observed species, (B) inversed Simpson, (C) Pielou's evenness and (D) Shannon over time in the two study groups.



Supplementary Figure 12. Difference in the microbiome composition (beta-diversity) between the placebo and probiotics group. (A) Group differences and (B) explained variance of moisture and subject. BH = Benjamini-Hochberg correction; R^2 = explained variance.



Supplementary Figure 13. Identified altered taxa in both study groups for all time points. The significance of the placebo and probiotic comparisons were carried out using Wilcoxon signed-rank test and the p-value adjusted using Benjamini-Hochberg method.

Supplementary Table 7. Explained variance of beta-diversity.

Variable	F-model	R^2	p	p-BH
Subject ID	4.556	0.809	0.001	0.006
Time*group	0.806	0.049	0.947	1.000
Moisture	3.861	0.045	0.001	0.006
Sex	2.293	0.027	0.003	0.013
Group	2.020	0.024	0.005	0.013
BMI	1.985	0.024	0.004	0.013
Age	1.760	0.021	0.019	0.041
Calprotectin	1.353	0.016	0.122	0.226
GSRS	1.135	0.014	0.276	0.448
BDI	0.974	0.012	0.475	0.685
Time point	0.446	0.011	1	1
HAM-D	0.742	0.009	0.847	1.000
STAI1	0.649	0.008	0.933	1.000

Notes. R^2 = explained variance; BH = Benjamini-Hochberg correction; GSRS = Gastrointestinal Symptom Rating Scale; BDI = Beck Depression Inventory; HAM-D = Hamilton Rating Scale for Depression; STAI1 = State-Trait Anxiety Inventory 1.

Supplementary Table 8. Changes in cortical thickness over time (ANOVA).

	Brain region	MNI			k	F_{max}	$p_{peak\ (FWE)}$	$p_{cluster\ (FWE)}$
		x	y	z				
	Main effect Group							
L	Inferior temporal gyrus	-55	-37	-23	36	18.00	0.231	0.444
R	Superior temporal gyrus	54	-14	-9	112	17.03	0.310	0.032
L	Superior frontal gyrus	-21	31	45	32	16.56	0.355	0.495
L	Precentral gyrus	-55	-7	20	75	16.03	0.411	0.124
R	Superior temporal gyrus	60	-39	10	71	15.47	0.477	0.143
L	Postcentral gyrus	-42	-29	39	59	14.05	0.661	0.217
L	Superior frontal gyrus	-21	7	50	17	13.80	0.694	0.702
L	Superior temporal gyrus	-66	-38	8	13	13.53	0.730	0.756
	Main effect Time							
R	Parahippocampal gyrus	21	-44	-11	7	12.90	0.807	0.830
	Interaction Time*group							
R	Lateral orbitofrontal gyrus	23	30	-12	71	21.23	0.083	0.143
L	Lingual gyrus	-5	-91	-5	10	13.17	0.775	0.794

Notes. R = right hemisphere; L = left hemisphere; *k* = cluster size in number of voxels; FWE = familywise error corrected.

Supplementary Table 9. Changes in gyrification over time (ANOVA).

	Brain region	MNI x	y	z	k	F_{max}	$p_{peak\ (FWE)}$	$p_{cluster\ (FWE)}$
	Main effect Group							
L	Middle frontal gyrus	-41	10	24	8	12.68	0.771	0.902
	Main effect Time							
L	Inferior temporal gyrus	-48	-7	-33	10	14.44	0.793	0.870
L	Insula	-39	-4	-15	10	14.33	0.806	0.870
	Interaction Time*group							
L	Central sulcus	-39	-16	35	28	14.93	0.733	0.474

Notes. L = left hemisphere; *k* = cluster size in number of voxels; FEW = familywise error corrected.

Supplementary Table 10. Changes in the sulcus depth over time (ANOVA).

	Brain region	MNI x	y	z	k	F_{max}	$P_{peak\ (FWE)}$	$p_{cluster\ (FWE)}$
	Main effect Group							
L	Medial superior frontal gyrus	-11	42	20	19	13.60	0.809	0.688
R	Middle frontal gyrus	26	3	50	24	13.67	0.802	0.596
L	Middle frontal gyrus	-35	23	50	10	15.78	0.530	0.839
L	Superior frontal gyrus	-21	-8	61	15	14.64	0.679	0.759
R	Supramarginal gyrus	36	-42	36	51	17.69	0.318	0.209
R	Superior occipital gyrus	13	-88	35	29	19.55	0.181	0.506
	Main effect Time							
	No suprathreshold clusters							
	Interaction Time*group							
	No suprathreshold clusters							

Notes. R = right hemisphere; L = left hemisphere; *k* = cluster size in number of voxels; FEW = familywise error corrected.

Supplementary Table 11. Activation changes over time in face processing in the probiotics group.

	Brain region	MNI x	y	z	k	T_{max}	$P_{peak(FWE)}$	$P_{cluster\ (FWE)}$
Changes over time in neutral faces								
Decreased activation								
R	Putamen, nucleus caudate	20	16	10	251	4.78	0.246	<0.001
L	Putamen, nucleus caudate, pallidum	-18	6	12	223	4.06	0.782	<0.001
Increased activation								
No suprathreshold clusters								
Changes over time in semi fearful faces								
No suprathreshold clusters at all								
Changes over time in fearful faces								
Decreased Activation								
L	Middle occipital gyrus	-20	-86	12	91	3.96	0.975	0.036
Increased activation								
No suprathreshold clusters								

Notes. R = right hemisphere; L = left hemisphere; *k* = cluster size in number of voxels; FWE = familywise error corrected.

Supplementary Table 12. Activation changes over time in face processing in the placebo group.

	Brain region	MNI x	y	z	k	T_{max}	$P_{peak\ (FWE)}$	$P_{cluster\ (FWE)}$
Changes over time in neutral faces								
Decreased activation								
No suprathreshold cluster								
Increased activation								
R	Cuneus, calcarine gyrus	8	-82	30	203	5.00	0.126	<0.001
Changes over time in semi fearful faces								
Decreased activation								
R	Inferior frontal gyrus	48	10	26	122	4.53	0.485	0.009
L	Inferior frontal gyrus	-42	20	24	105	5.00	0.126	0.019
R	Middle occipital gyrus, angular gyrus	30	-68	34	202	4.34	0.595	<0.001
Increased Activation								
L	Superior frontal gyrus, anterior cingulum	-10	56	16	98	4.05	0.268	0.026
R	Cuneus, superior occipital gyrus	10	-82	30	85	4.48	0.942	0.048
Changes over time in fearful faces								
Decreased Activation								
No suprathreshold clusters								
Increased activation								
L	Middle cingular cortex	-6	-26	32	199	5.05	0.105	<0.001
R	Cuneus, calcarine sulcus	10	-82	30	211	5.45	0.026	<0.001

Notes. R = right hemisphere; L = left hemisphere; *k* = cluster size in number of voxels; FWE = familywise error corrected.

Supplementary Table 13. Patients versus healthy controls during neutral face processing.

	Brain region	MNI x	MNI y	MNI z	k	T_{max}	$P_{peak\ (FWE)}$	$P_{cluster\ (FWE)}$
	Patients > Healthy controls							
B	Medial superior frontal gyrus, anterior cingulate gyrus	4	28	42	74	7.14	<0.001	<0.001
R	Amygdala, putamen	22	4	-10	21	6.31	<0.001	<0.001
L	Putamen, nucleus caudate	-18	2	12	31	7.07	<0.001	<0.001
L	Rolandic operculum, insula	-36	-6	16	17	6.53	<0.001	<0.001
L	Supramarginal gyrus	-58	-22	40	15	6.07	<0.001	<0.001
L	Cerebellum, fusiform gyrus	-34	-42	-24	25	6.34	<0.001	<0.001
R	Fusiform gyrus	46	-62	-28	19	6.73	<0.001	<0.001
R	Fusiform gyrus, cerebellum	26	-82	-16	17	6.43	<0.001	<0.001
		38	-52	-22	15	6.09	<0.001	<0.001
R	Cerebellum superior posterior lobe	26	-56	-20	27	6.70	<0.001	<0.001
		34	-70	-20	33	6.51	<0.001	<0.001
L	Cerebellum superior posterior lobe	-32	-60	-30	171	7.42	<0.001	<0.001
L	Cerebellum	-6	-80	-32	30	6.35	<0.001	<0.001
	Healthy controls > Patients							
L	Precentral gyrus	-28	-22	62	71	8.23	<0.001	<0.001
R	Lingual gyrus	10	-76	-2	72	7.56	<0.001	<0.001
R	Calcarine sulcus	12	-88	12	17	6.58	<0.001	<0.001

Notes. R = right hemisphere; L = left hemisphere; B = bilateral; *k* = cluster size in number of voxels; FEW = familywise error corrected.

Supplementary Table 14. Patients versus healthy controls during semi-fearful face processing.

	Brain region	MNI			k	T_{max}	$P_{peak\ (FWE)}$	$P_{cluster\ (FWE)}$
		x	y	z				
	Patients > Healthy Controls							
L	Rolandic operculum	-40	-8	16	11	5.93	0.015	0.001
	Healthy controls > Patients							
R	Precentral gyrus	16	-26	56	65	6.76	0.001	<0.001
L	Precentral gyrus	-28	-24	56	284	9.20	<0.001	0.026
L	Middle cingulate gyrus, paracentral lobule	-8	-30	50	26	7.14	<0.001	<0.001
R	Lingual gyrus, calcarine gyrus	8	-78	0	168	9.47	<0.001	<0.001
L	Lingual gyrus	-8	-76	-4	29	6.53	0.002	<0.001
R	Superior occipital gyrus	24	-80	24	14	6.06	0.01	<0.001
R	Calcarine gyrus, cuneus	8	-90	8	43	7.19	<0.001	0.026
L	Cuneus, superior occipital gyrus	-8	-96	16	29	6.12	0.008	<0.001

Notes. R = right hemisphere; L = left hemisphere; *k* = cluster size in number of voxels; FWE = familywise error corrected.

Supplementary Table 15. Patients versus healthy controls during fearful faces processing.

	Brain region	MNI			k	T_{max}	$P_{peak\ (FWE)}$	$P_{cluster\ (FWE)}$
		x	y	z				
Patients > Healthy Controls								
L	Cerebellum, fusiform gyrus	-40	-54	-22	26	6.23	0.006	<0.001
Healthy controls > Patients								
L	Precentral gyrus	-28	-22	62	77	7.28	<0.001	<0.001
R	Lingual gyrus	10	-78	-2	52	7.37	<0.001	<0.001
R	Calcarine gyrus	10	-90	10	12	6.12	<0.001	<0.001

Notes. R = right hemisphere; L = left hemisphere; *k* = cluster size in number of voxels; FWE = familywise error corrected.

Supplementary References

1. World Health Organization. WHO Collaborating Centre for Drug Statistics Methodology: ATC classification index with DDDs, 2020. 2019.

2. Gupta SK. Intention-to-treat concept: a review. *Perspectives in clinical research* 2011; **2**(3)**:** 109.

3. Cramer JA, Rosenheck R. Compliance with medication regimens for mental and physical disorders. *Psychiatric Services* 1998; **49**(2)**:** 196-201.

4. Kowarik A, Templ M. Imputation with the R Package VIM. *Journal of Statistical Software* 2016; **74**(7)**:** 16.

5. Bates D, Machler M, Bolker BM, Walker SC. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 2015; **67**(1)**:** 1-48.

6. Riedel M, Moller HJ, Obermeier M, Schennach-Wolff R, Bauer M, Adli M *et al.* Response and remission criteria in major depression - A validation of current practice. *Journal of Psychiatric Research* 2010; **44**(15)**:** 1063-1068.

7. Falony G, Joossens M, Vieira-Silva S, Wang J, Darzi Y, Faust K *et al.* Population-level analysis of gut microbiome variation. *Science* 2016; **352**(6285)**:** 560-564.

8. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* 2016; **13**(7)**:** 581-+.

9. Vandeputte D, Kathagen G, D'Hoe K, Vieira-Silva S, Valles-Colomer M, Sabino J *et al.* Quantitative microbiome profiling links gut community variation to microbial load. *Nature* 2017; **551**(7681)**:** 507-+.

10. Prest EI, Hammes F, Kötzsch S, van Loosdrecht MC, Vrouwenvelder JS. Monitoring microbiological changes in drinking water systems using a fast and reproducible flow cytometric method. *Water Res* 2013; **47**(19)**:** 7131-7142.

11. Kurilshikov A, Medina-Gomez C, Bacigalupe R, Radjabzadeh D, Wang J, Demirkan A *et al.* Large-scale association analyses identify host factors influencing human gut microbiome composition. *Nature Genetics* 2021; **53**(2)**:** 156-+.

12. Zhang XY, Yi NJ. NBZIMM: negative binomial and zero-inflated mixed models, with application to microbiome/metagenomics data analysis. *Bmc Bioinformatics* 2020; **21**(1).

13. Mugler III JP, Brookeman JR. Three-dimensional magnetization-prepared rapid gradient-echo imaging (3D MP RAGE). *Magnetic resonance in medicine* 1990; **15**(1)**:** 152-157.

14. Gaser C, Dahnke R. CAT-a computational anatomy toolbox for the analysis of structural MRI data. *HBM* 2016; **2016:** 336-348.

15. Filkowski MM, Haas BW. Rethinking the Use of Neutral Faces as a Baseline in fMRI Neuroimaging Studies of Axis-I Psychiatric Disorders. *Journal of Neuroimaging* 2017; **27**(3)**:** 281-291.